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Resistance of different types of nontuberculos mycobacteria to aldehyde disinfectants

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ABSTRACT

Nontuberculos mycobacteria (NTM) are widely distributed, and investigation of their ecology establishes a basis for the development of new agents and methods for their identification, differentiation and devitalization. This study aimed to evaluate the resistance of different types of NTM to aldehyde-containing disinfectants with different concentrations of active ingredients. The experiments employed complex disinfectants with glutaraldehyde as the active ingredient. Test cultures of several types of NTM belonging to groups I (*M. kansasii*), II (*M. gordonae, M. scrofulaceum*), III (M. *intracellulare, M. terrae, M. triviale, M. xenopi*) and IV (*M. diernhoferi, M. flavescens, M. fortuitum, M. phlei, M. smegmatis, M. thamnopheos*) according to the Runyon classification, were used. The research confirmed that NTM of different species and strains show different resistance to the same disinfectant. The mycobacteria species *M. fortuitum, M. intracellulare, M. scrofulaceum,* and *M. thamnopheos* displayed the highest resistance to aldehyde-containing disinfectants. The effectiveness of an antimicrobial agent against mycobacteria depends on the concentration of the active ingredient and the duration of exposure. Future research should be focused on examining variations in the resistance of mycobacteria to new disinfectants from different chemical categories.

Key words: disinfectant; nontuberculos mycobacteria (NTM); resistance; bactericidal effect; concentration; exposure

Introduction

Despite the fact that the study of NTMs began after Robert Koch's discovery of the causative agent of tuberculosis, their clinical and epidemiological significance has long been underestimated. This group of mycobacteria includes a large number of species that are widely distributed in the environment (PARRISH, 2019; ARMSTRONG and PARRISH, 2021). More than 278 different species of NTM are known (ICSP, 2023).

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NTMs are saprophytes, common inhabitants of various environments (TO et al., 2020). They are characterized by exceptional adaptability and durability. They are capable of colonization and survival even in very unfavorable conditions (PAVLIK et al., 2022).

NTMs were previously considered non-pathogenic for humans and animals, and until the 1950s, there were only isolated reports of cases of diseases caused by these microorganisms in the world. In recent years, the number of infections caused by fast-growing mycobacteria has increased markedly worldwide. They are usually associated with trauma, surgery, and disseminated infections (CARDOZO LOMAQUIZ et al., 2021). The most common NTMs that cause most infections in humans are the *Mycobacterium avium intracellulare*, *M. kansasii, M. marinum, M. ulcerans, M. abscessus, M. chelonae,* and *M. fortuitum* complex. These organisms are most commonly found in soil, indoor and outdoor water sources, and are known to colonize poorly disinfected medical equipment (WINBURN and SHARMAN, 2023).

Due to their cell wall composition and ability to adapt, mycobacteria can survive for years in various habitats (HRUSKA and KAEVSKA, 2012). Nineteen species of mycobacteria have been isolated from soil, including 7 (36.8%) fast-growing mycobacteria and 12 (63.2%) slow-growing mycobacteria (HU et al., 2017). Other researchers have reported that mycobacteria and lineages within a group often showed predictable preferences for specific environmental conditions. Soil contains a large amount of previously undescribed mycobacterial diversity, and lineages that include known pathogens have rarely been detected in soil (WALSH et al., 2019). It has been proven that the distribution of mycobacteria in soil is influenced by its type and chemical composition (GLICKMAN et al., 2020; PARSONS et al., 2022). Thus, forty-eight isolates of NTM were detected, of which 25.3% were from soil samples, 11.8% from water, and 9.1% from animal feces samples. Soils around water sources were the most contaminated (29.8%). The most frequently detected mycobacteria in these samples were *M. fortuitum-peregrinum* complex, *M. avium* complex, *M. gordonae*, and *M. nonchromogenicum* (KANKYA et al., 2011). According to other data, the most common mycobacteria are *M. fortuitum* (19.8%) and *M. flavescens* (16.8%) (KHALEDI et al., 2016).

The presence of mycobacteria in plant tissues has been established, which in turn raises concerns about their possible transmission to animals and humans (HRUSKA and KAEVSKA, 2012).

The problem of NTM has become an acute issue in practical veterinary medicine. When animals are infected with these microorganisms, they become sensitized to tuberculin, resulting in parallergic reactions, which complicates the diagnosis of tuberculosis infection, and also leads to economic losses in cases of the unjustified slaughter of such animals (ZAVGORODNII et al., 2021; FERNÁNDEZ-VEIGA et al., 2023). It has been proven that the predominant species are mycobacteria of the *M. avium* complex (CLARKE et al., 2022). Other researchers believe that the most common species are *M. fortuitum* (43.9%), as well as *M. novocastrense, M. terrae, M. flavescens, M. holsaticum, M. cosmeticum, M. virginiense, M. intracellulare, M. mageritense, M. minnesotensis, M. duvalii, M. lehmannii*, and *M. koreense* (TINGAN et al., 2022).

The basis for their widespread distribution in soils, natural and anthropogenic waters is primarily their resistance to disinfectants, biofilm formation, and their ability to adapt to changing environmental conditions (FALKINHAM, 2021).

The situation with the spread and pathogenic effects of NTM is predicted to worsen, since, like the causative agents of tuberculosis, they are highly naturally resistant and quickly acquire resistance to antimicrobial agents (SAXENA et al., 2021). It has been reported that some mycobacterial species have developed resistance to disinfectants by adjusting their lipid composition and net surface charge (DE CARVALHO et al., 2020).

An integrated approach to the search for and use of new disinfectants, taking into account the dynamics of the death of bacteria under the influence of different disinfectants, makes it possible to improve disinfection regimes and schemes, as well as to conduct quality control of the entire complex of anti-epizootic measures (PALIY, 2018).

The issue of NTM resistance as atypical mycobacteria to disinfectants that are widely used in veterinary practice remains relevant.

The aim of this paper is to study the resistance of different types of NTM to aldehyde-containing disinfectants.

Materials and methods

The research was conducted using complex disinfectants with the active ingredient glutaraldehyde:

Agent 1: glutaraldehyde - 11.0%, a mixture of quaternary ammonium compounds - 25.0%, isopropyl alcohol, non-ionic surfactants, water.

Agent 2: glutaraldehyde - 21.5%, a mixture of quaternary ammonium compounds - 10.5%, surfactants, water softener, water.

The disinfectants were used at concentrations of 2.0, 2.5, 3.0, 3.5% and 0.5, 1.0, 1.5, 2.0%, respectively, for exposures of 1, 5, 24 hours according to the current guidelines for testing disinfectants in agriculture (WALES et al., 2021).

Test cultures of different types of NTMs belonging to groups I, II, III and IV according to the Runyon classification (1959) were used in the experiments (Table 1).

Table 1. NTM used in the experiments

Species	Passport data
Group I – photochromogenic mycobacteria	
 *M. kansasii*	Strain 11-P (1), obtained from the Odesa Institute of Biochemistry and Sanitation in 1998, is a museum strain, non-pathogenic for laboratory and farm animals
Group II – scotochromogenic mycobacteria	
 *M. scrofulaceum*	Strain 31-82, obtained by the Laboratory of Tuberculosis Study of the IECVM UAAS from the reference culture by selection in 1982, is a production strain, non-pathogenic for farm and laboratory animals
 *M. gordonae*	Inventory No. 47, received from the Zaporizhzhia Regional Veterinary Laboratory in 1998, is a museum strain, non-pathogenic for farm and laboratory animals

Table 1. NTM used in the experiments (continued)

Species	Passport data
Group III – nonphotochromogenic mycobacteria	
 *M. intracellulare*	Strain 78-98, obtained by the Laboratory of Tuberculosis Study of the IECVM UAAS from the reference culture by selection in 1998, is a production, non-pathogenic strain for farm and laboratory animals
 *M. terrae*	Inventory No. 1450, received from the Tarasevych State Research Institute for Standardization and Control of Medical Biological Drugs in 1995, is a museum strain, non-pathogenic for laboratory and farm animals
 *M. triviale*	Inventory No. 59, received from the Kharkiv Regional Veterinary Laboratory in 1984, is a museum strain, non-pathogenic for farm and laboratory animals
 *M. xenopi*	Inventory No. without a number, received from the Tarasevych State Research Institute for Standardization and Control of Medical Biological Preparations in 1995, is a museum strain, non-pathogenic for laboratory and farm animals
Group IV – fast-growing mycobacteria	
 *M. phlei*	Inventory No. 22, received from the All-Union Institute of Experimental Veterinary Medicine in 1990, is a museum strain, non-pathogenic for farm and laboratory animals
 *M. diernhoferi*	Inventory No. 64, obtained in 1990 from the lymph nodes of cattle, Luhansk region, is a museum strain, non-pathogenic for farm and laboratory animals

Table 1. NTM used in the experiments (continued)

Species	Passport data
 *M. fortuitum*	Strain 122, obtained from the Tarasevych State Research Institute for Standardization and Control of Medical Biological Products in 1995, is a museum strain, non-pathogenic for laboratory and farm animals
 *M. flavescens*	Inventory No. 119, obtained in 2008 from pathological material from cattle, Kyiv region, Vasylkiv district, is a museum strain, non-pathogenic for farm and laboratory animals
 *M. smegmatis*	Inventory No. 51, obtained in 1986 from soil, Kharkiv region, is a museum strain, non-pathogenic for farm and laboratory animals
 *M. thamnopheos*	Inventory No. 77, obtained in 1984 from bronchial mucus of cattle, Kherson region, is a museum strain, non-pathogenic for laboratory and farm animals

Mycobacteria were grown on potatoes with glycerol for 14-21 days (depending on the species) at 37.0±0.5°C (PRADHAN et al., 2021). All test cultures of mycobacteria had typical culture and biological properties.

In order to conduct the experiment, the first step was to prepare a suspension of test cultures of mycobacteria, each separately, at a concentration of $2x10^9$ bacterial cells in 1.0 cm^3 of isotonic solution. For this purpose, the bacterial mass of the mycobacterial test cultures was transferred by bacteriological loop into sterile 200 cm^3 vials with beads, previously weighed on a laboratory precision scale. After that, the mass of mycobacteria introduced into them was determined by weighing. Then the required volume of sterile isotonic solution was added. In order to obtain a homogeneous suspension of mycobacteria, the vials were shaken on a shaker for 30 minutes.

The next stage of the research was the preparation of working solutions of disinfectants in the above concentrations. After that, 0.2 cm^3 of suspensions of the respective mycobacterial species were added separately to each 10 cm^3 vial of disinfectant. The contents of the vials were thoroughly mixed and given the appropriate exposure.

As the control for the bactericidal effect of the test drug, a vial with a suspension of test cultures of mycobacteria was used, into which 10 cm^3 of sterile isotonic solution was added instead of disinfectant solutions.

At the final stage, samples of 10 cm^3 were taken from the experimental and control vials, transferred to centrifuge tubes, which were centrifuged at 3000 rpm for 30 minutes. To stop the action of the agents in the test tubes, the precipitate formed after centrifugation, as well as the control sample, were washed twice with sterile isotonic solution by centrifugation.

The resulting suspension was inoculated onto a nutrient medium for cultivation of mycobacteria (PALANGE et al., 2016). The tubes with the cultures were placed in a thermostat at 37.0±0.5°C and the growth of the cultures was recorded every 3 to 5 days for 90 days.

The relative resistance coefficient was used as a parameter for assessing the level of resistance of mycobacterial cultures.

The coefficient of relative resistance (F) of cultures is an indicator obtained by dividing the concentration of the drug (C), which causes 100% death of the culture under study, by the concentration of the same drug that ensures the destruction of the reference culture (C_e) within the same period of time (PALIY, 2018).

The calculations were based on data from the study of the bactericidal properties of disinfectants by the suspension method when used at different concentrations and exposures. Mycobacteria of the *M. fortuitum* species were used as a reference test culture.

Table 2. Bactericidal properties of disinfectant No. 1 against NTM of groups I, II and III according to the Runyon classification

Culture	Exposure	Concentration				Control
		2.0%	2.5%	3.0%	3.5%	
M. kansasii	1 hour	+	+	+	–	+
	5 hours	+	+	–	–	+
	24 hours	+	–	–	–	+
M. gordonae	1 hour	+	+	+	–	+
	5 hours	+	+	–	–	+
	24 hours	+	–	–	–	+
M. scrofulaceum	1 hour	+	+	+	–	+
	5 hours	+	+	+	–	+
	24 hours	+	–	–	–	+
M. intracellulare	1 hour	+	+	+	–	+
	5 hours	+	+	+	–	+
	24 hours	–	–	–	–	+
M. terrae	1 hour	+	+	–	–	+
	5 hours	+	+	–	–	+
	24 hours	+	–	–	–	+
M. triviale	1 hour	+	+	+	–	+
	5 hours	+	+	+	–	+
	24 hours	+	–	–	–	+
M. xenopi	1 hour	+	+	–	–	+
	5 hours	+	+	–	–	+
	24 hours	–	–	–	–	+

Notes: "–" – absence of mycobacterial growth; "+" – presence of mycobacterial growth

The calculations were made using the formula:

$$F = \frac{C}{C_{å}}$$

where F is the coefficient of relative resistance of the tested cultures;

– C is the concentration of aqueous solutions of the drug that causes 100% death of the test culture, %;

– C_e is the concentration of aqueous solutions of the drug that causes 100% death of the reference culture, %.

To summarize the data obtained, we used the calculation of the value of the variation trait, which was calculated as the sum of the values of the trait in individual units of the population - the arithmetic mean (μ).

When analyzing the data obtained, it was noted that if the value of the relative resistance of the studied mycobacteria is equal to 1, then this culture is equal to the reference test culture in terms of resistance to the disinfectant, and if this indicator is less than 1, then this culture was considered less resistant to the disinfectant than *M. fortuitum*.

Results

The results of the experiment to determine the resistance of NTMs to disinfectant No. 1 are shown in Table 2 and 3.

Table 3**.** Bactericidal properties of drug No. 1 against NTMs of group IV according to the Runyon classification

Culture	Exposure	Concentration				Control
		2.0%	2.5%	3.0%	3.5%	
M. diernhoferi	1 hour	+	+	–	–	+
	5 hours	+	+	–	–	+
	24 hours	–	–	–	–	+
M. flavescens	1 hour	+	+	+	–	+
	5 hours	+	+	+	–	+
	24 hours	+	–	–	–	+
M. fortuitum	1 hour	+	+	+	–	+
	5 hours	+	+	+	–	+
	24 hours	+	–	–	–	+
M. phlei	1 hour	+	+	+	–	+
	5 hours	+	–	–	–	+
	24 hours	+	–	–	–	+
M. smegmatis	1 hour	+	+	+	–	+
	5 hours	+	+	–	–	+
	24 hours	–	–	–	–	+
M. thamnopheos	1 hour	+	+	+	–	+
	5 hours	+	+	+	–	+
	24 hours	+	–	–	–	+

Notes: "–" – absence of mycobacterial growth; "+" – presence of mycobacterial growth

The analysis of the research results obtained (Table 2) showed that the use of this drug in the regimens of 2.5% - 24 h, 3.0% - 5-24 h and 3.5% - 1-24 h led to the disinfection of *M. kansasii*. The bactericidal effect of the drug after its action on *M. gordonae* was observed when it was used at concentrations of 2.5% (24 h), 3.0% (5-24 h) and 3.5% (1-24 h). The bactericidal effect on *M. scrofulaceum* and *M. triviale* was observed when the disinfectant was used at concentrations of 2.5-3.0% (24 hours) and 3.5% (1-24 hours). *M. terrae* and *M. xenopi* had almost the same resistance to the disinfectant. Thus, these test cultures died when exposed to the disinfectant at a concentration of 3.0-3.5% for 1-24 h and at a concentration of 2.5% for 24 h, and *M. xenopi* also died when the drug was applied at a concentration of 2.0% for 24 h. The effect of the product at a concentration of 2.0-3.0% with exposure for up to 24 h caused complete devitalization of *M. intracellulare*, as well as after exposure to it at a concentration of 3.5% (1-24 h).

It was found that the action of a 2.0-2.5% solution of the drug for 24 h and 3.0-3.5% (1-24 h) caused decontamination of *M. diernhoferi*. The bactericidal properties of this disinfectant against *M. flavescens, M. fortuitum, M. thamnopheos* were found at a concentration of 2.5-3.0% at 24 h exposure and 3.5% at 1-24 h. The growth of *M. phlei* was absent after exposure to the drug at concentrations of 2.5-3.0% (5-24 h) and 3.5% (1-24 h), and *M. smegmatis* died after treatment with this drug in the following regimens: 2.0-2.5% - 24 hours; 3.0% - 5-24 hours; 3.5% - 1-24 hours.

The results of the experiment to determine the resistance of NTM to disinfectant No. 2 are shown in Table 4 and 5.

It was found that the culture *M. kansasii* loses its viability after exposure to the product at a concentration of 2.0% (1-24 h). Devitalization of *M. gordonae* was observed when the drug was used in the following regimens: 0.5% - 24 hours; 1.0-1.5% - 5-24 hours; 2.0% - 1-24 hours. The bactericidal effect of the disinfectant on the development of *M. scrofulaceum* was found at concentrations of 1.5% (24 h) and 2.0% (1-24 h). The culture *M. intracellulare* died after exposure to the product at a concentration of 2.0% (5-24 h), and the culture *M. terrae* lost its viability when exposed to the disinfectant at concentrations of 1.0% (24 hours) and 1.5-2.0% (5-24 hours). Mycobacteria *M. triviale* were inactivated after exposure to the product at a concentration of 0.5-1.0% for 24 hours, and at a concentration of 1.5-2.0% for 1-24 hours, as were *M. xenopi* under the same regimens of application of the product and additionally at a concentration of 1.0% (5 hours).

Thus, the culture *M. diernhoferi* was inactivated when the drug was used in the following regimens: 1.0-1.5% for 24 h exposure and 2.0% for 5-24 h. No growth of *M. flavescens* colonies was observed after exposure to the drug at concentrations of 0.5% - 24 h; 1.0% - 5-24 h and 1.5-2.0% at exposure for 1-24 h. The drug causes devitalization of *M. fortuitum* at a concentration of 2.0% for 5-24 hours. The disinfectant at a concentration of 1.5-2.0% at an exposure for 5-24 h had a bactericidal effect on the *M. phlei* culture. Mycobacteria *M. smegmatis* were killed by the action of this drug at concentrations of 0.5-1.0% (24 h); 1.5-2.0% (5-24 h). The bactericidal effect on *M. thamnopheos* was found at a concentration of 1.5 % (24 h) and 2.0 % (1-24 h).

Calculations were conducted to determine the coefficient of the relative resistance of mycobacterial cultures to aldehyde disinfectants (Table 6).

As a result of these calculations, it was determined (Table 6) that certain species of mycobacteria show different levels of resistance to the same disinfectant. Almost all the tested species, except for *M. terrae, M. xenopi, M. diernhoferi*, showed high resistance to the effect of preparation No. 1 at exposure for 1 h. With the increase of exposure to 5 h, *M. kansasii, M. gordonae, M. terrae, M. xenopi, M. diernhoferi, M. phlei, M. smegmatis* were less resistant to the drug than *M. fortuitum*. The highest resistance together with the reference culture to the bactericidal effect of the disinfectant at 24 h exposure was determined in *M. kansasii, M. gordonae, M. scrofulaceum, M. terrae, M. triviale, M. flavescens, M. phlei* and *M. thamnopheos*.

At the same time, the highest resistance to the effect of drug No. 2, after 1 h in comparison with *M. fortuitum* was found in *M. intracellulare, M. terrae, M. diernhoferi, M. phlei, M. smegmatis*; when the drug was exposed for 5 h, this indicator was highest in the mycobacteria *M. kansasii, M. scrofulaceum, M. intracellulare, M. diernhoferi, M. thamnopheos*, while the highest resistance to the bactericidal effect of the disinfectant at 24 h exposure was found in only *M. kansasii* and *M. intracellulare* cultures.

When analyzing the average statistical index, it was found that *M. scrofulaceum, M. triviale, M. flavescens, M. fortuitum, M. thamnopheos* were the most resistant to drug No. 1, and *M. fortuitum* and *M. intracellulare* showed the highest resistance to drug No. 2.

Table 4. Bactericidal properties of agent No. 2 against NTMs of groups I, II and III according to the Runyon classification

Culture	Exposure	Concentration				Control
		0.5%	1.0%	1.5%	2.0%	
M. kansasii	1 hour 5 hours 24 hours	+ + +	+ + +	+ + +	– – –	+ + +
M. gordonae	1 hour 5 hours 24 hours	+ + –	+ – –	+ – –	– – –	+ + +
M. scrofulaceum	1 hour 5 hours 24 hours	+ + +	+ + +	+ + –	– – –	+ + +
M. intracellulare	1 hour 5 hours 24 hours	+ + +	+ + +	+ + +	+ – –	+ + +
M. terrae	1 hour 5 hours 24 hours	+ + +	+ + –	+ – –	+ – –	+ + +
M. triviale	1 hour 5 hours 24 hours	+ + –	+ + –	– – –	– – –	+ + +
M. xenopi	1 hour 5 hours 24 hours	+ + –	+ – –	– – –	– – –	+ + +

Notes: "–" – absence of mycobacterial growth; "+" – presence of mycobacterial growth

Table 5. Bactericidal properties of agent No. 2 against NTMs of group IV according to the Runyon classification

Culture	Exposure	Concentration				Control
		0.5%	1.0%	1.5%	2.0%	
M. diernhoferi	1 hour 5 hours 24 hours	+ + +	+ + –	+ + –	+ – –	+ + +
M. flavescens	1 hour 5 hours 24 hours	+ + –	+ – –	– – –	– – –	+ + +
M. fortuitum	1 hour 5 hours 24 hours	+ + +	+ + +	+ + +	+ – –	+ + +
M. phlei	1 hour 5 hours 24 hours	+ + +	+ + +	+ – –	+ – –	+ + +
M. smegmatis	1 hour 5 hours 24 hours	+ + –	+ + –	+ – –	+ – –	+ + +
M. thamnopheos	1 hour 5 hours 24 hours	+ + +	+ + +	+ + –	– – –	+ + +

Notes: "–" – absence of mycobacterial growth; "+" – presence of mycobacterial growth

Table 6. Coefficient of the relative resistance of mycobacterial cultures to aldehyde disinfectants

Mycobacteria culture	Disinfectant							
	No 1				No 2			
	1 h	5 h	24 h	μ	1 h	5 h	24 h	μ
M. kansasii	1	0.83	1	0.94	0.8	1	1	0.93
M. gordonae	1	0.83	1	0.94	0.8	0.5	0.25	0.52
M. scrofulaceum	1	1	1	1	0.8	1	0.75	0.85
M. intracellulare	1	1	0.75	0.92	1	1	1	1
M. terrae	0.83	0.83	1	0.89	1	0.75	0.5	0.75
M. triviale	1	1	1	1	0.6	0.75	0.25	0.53
M. xenopi	0.83	0.83	0.75	0.8	0.6	0.5	0.25	0.45
M. diernhoferi	0.83	0.83	0.75	0.8	1	1	0.5	0.83
M. flavescens	1	1	1	1	0.6	0.5	0.25	0.45
M. phlei	1	0.66	1	0.89	1	0.75	0.75	0.83
M. smegmatis	1	0.83	0.75	0.86	1	0.75	0.25	0.67
M. thamnopheos	1	1	1	1	0.8	1	0.75	0.85
M. fortuitum	1	1	1	1	1	1	1	1

Discussion

Changes in climate and the ecological niches of circulation of pathogens in humans and farm animals have led to the emergence of adaptive mechanisms in microorganisms in accordance with the conditions of their existence (BOGACH et al., 2020; TAN et al., 2022). The widespread and ubiquitous distribution of pathogens in the environment requires the development of effective means and methods for their eradication, taking into account scientific data and modern regulations (PALIY et al., 2019; DAI et al., 2023).

Several features, such as biofilm formation and the ability of selected NTM species to form different colony morphotypes, may play a role in their pathogenesis that is not observed in the related, well-characterized pathogen *M. tuberculosis* (CLAEYS and ROBINSON, 2018).

The characteristics of NTMs, including resistance to disinfectants, adherence to surfaces, and biofilm formation, pose challenges to current cleaning and disinfection procedures (FALKINHAM, 2021). At the same time, it has been shown that drinking untreated water and living in close contact with cattle or other domestic animals can be risk factors for the possibility of infecting humans and animals with NTMs hrough these ecosystems (KANKYA et al., 2011). The importance of modern disinfection is also indicated by the fact that surgical infections caused by the pathological effects of NTM can be prevented by proper sterilization of instruments (CHAUDHURI et al., 2010; BENTO et al., 2020). In Europe, chemical disinfectants are tested for their tuberculocidal and mycobactericidal efficacy according to the internationally accepted testing procedure described in EN 14348 (BRILL et al., 2021).

M. avium and *M. intracellulare* were found to be many times more resistant to chlorine, chloramine, chlorine dioxide and ozone than other waterborne microorganisms (FALKINHAM, 2002). The analysis of the distribution of mycobacterial cultures by the average values of their resistance to chlorine disinfectant solutions at a fixed exposure showed that mycobacteria of the species *M. scrofulaceum, M. intracellulare, M. fortuitum, M. avium* have the highest resistance (PALIY, 2018).

Other researchers also argue that the bactericidal effect of different disinfectants varies depending on the *Mycobacterium* spp. Hydrogen peroxide has been shown to be less effective than unbuffered chlorine bleach (CHANG et al., 2015). Mycobacterial resistance to disinfectants was found to be independent of resistance to antimicrobial drugs (Shinoda et al., 2016). Other researchers suggest that *M. gordonae* is more resistant than other mycobacteria to chlorine in nutrient-poor media, and that increasing the temperature (from 4°C to 25°C) and lowering the pH results in better inactivation (LE DANTEC et al., 2002). *M. bolletii* and *M. massiliense* have been shown to be more resistant to acetic acid than *M. tuberculosis* (CORTESIA et al., 2014). NTMs have been shown to be more resistant to modern disinfectants than tuberculosis pathogens (LEE et al., 2022).

Recent studies have shown that *M. vaccae* cells have adapted to antimicrobial compounds, mainly by adjusting their lipid composition and net surface charge (DE CARVALHO et al., 2020).

Amine derivatives (glucoprotamine) have been reported to have high activity against *M. smegmatis, M. avium, M. kansasii, M. terrae* and *M. xenopi*. However, they are less effective against *M. chelonae* (TARASHI et al., 2022).

Clinical isolates of mycobacteria have been shown to be more resistant to chemical compounds compared to museum cultures (HERNÁNDEZ et al., 2005). The use of aldehyde disinfectants should be scientifically justified, because mycobacteria can survive their exposure and pose a risk of cross-infection for patients (FISHER et al., 2012).

Antibiograms are important for the targeted treatment of mycobacterial infections (PINTO-GOUVEIA et al., 2015). Therefore, when disinfecting, it is necessary to determine the level of mycobacterial resistance to the disinfectant used. It is believed that the resistance of pathogens is more related to the general mechanisms of adaptation of the pathogens to adverse environmental factors than to the widespread use of antimicrobial agents (YAGNUK et al., 2023).For example, resistance of mycobacteria to the same disinfectant has been

shown to vary within the same species, which should be considered in the development and use of antimicrobial agents.

Conclusions

NTMs of different species and strains show different levels of resistance to the same disinfectant. It was found that the most resistant to the action of disinfectants containing aldehydes are mycobacteria of the species *M. fortuitum, M. intracellulare, M. scrofulaceum,* and *M. thamnopheos*. The susceptibility of mycobacteria to an antimicrobial agent depends on its active ingredient content and exposure.

Improvement of disinfection regimens and schemes, control of their quality in the overall complex of antiepizootic measures is only possible with an integrated approach to the search for and use of new antimicrobial agents, taking into account the dynamics of bacterial death under their influence.

Declaration of competing interest

No potential conflict of interest was reported by the authors

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PALIY, A., A. ZAVGORODNII, K. RODIONOVA, S. BOROVKOV, O. PAVLICHENKO, R. DUBIN , T. IHNATIEVA: Rezistencija različitih tipova netuberkuloznih mikobakterija na aldehidne dezinficijense. Vet. arhiv 94, 499-512, 2024.

SAŽETAK

Netuberkulozne bakterije (NTM) široko su rasprostranjene, a istraživanje njihove ekologije stvara osnovu razvoja novih sredstava i metoda za identifikaciju, diferencijaciju i bakteriostazu navedene skupine mikroba. Cilj je istraživanja bio procijeniti rezistenciju više tipova NTM-a na aldehidne dezinficijense s različitim koncentracijama aktivnih sastojaka. Primjenjeni su složeni dezinficijensi s glutaraldehidom kao aktivnim sastojkom. Analizirana je kultura nekoliko tipova NTM-a koji su u skladu s Runyonovom klasifikacijom razvrstani u skupinu I (*M. kansasii*), skupinu II (*M. gordonae, M. scrofulaceum*), skupinu III (M. *intracellulare, M. terrae, M. triviale, M. xenopi*) i skupinu IV (*M. diernhoferi, M. flavescens, M. fortuitum, M. phlei, M. smegmatis, M. thamnopheos*). Istraživanje potvrđuje da različite vrste i sojevi NTM-a pokazuju različit stupanj rezistencije na isti dezinficijens. Vrste mikobakterija *M. fortuitum*, *M. intracellulare*, *M. scrofulaceum* i *M. thamnopheos* pokazuju visok stupanj rezistencije na dezinficijense koji sadržavaju aldehid. Učinkovitost antimikrobnih tvari protiv mikobakterija ovisi o koncentraciji aktivnog sastojka i vremeskom trajanju izloženosti dezinficijensu. Buduća bi se istraživanja trebala usredotočiti na analizu varijacija u rezistenciji mikobakterija na nove dezinficijense koji dolaze iz kemijski različitih skupina.

Ključne riječi: dezinficijensi; netuberkulozne mikobakterije (NTM); rezistencija; bakteriostatski učinak; koncentracija; izloženost