UDC 616 - 002.5 : 615.28+576.852.2

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ASSESSMENT OF THE EFFICACY OF STANDARD DISINFECTION REGIMES USED IN TUBERCULOSIS CLINIC

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We assessed the efficacy of several disinfectants accepted for use in tuberculosis institutions, by activity against *M.tuberculosis* cultures. Disinfectant test procedure is based on procedures adopted from a well-known method commonly used in disinfectant testing practice, which consists in submerging coarse calico test-objects contaminated with the test-microbes into a disinfectant. Analysis of the study results showed that both museum and clinical test-strains of *M.bovis* and *M.tuberculosis* are more pertinent to real tuberculosis causative microorganisms in terms of resistance to disinfectants. The study findings support the feasibility of using a number of various test-strains in the development of national tuberculocidal disinfectant regimens instead of "*Mycobacterium B-5*" alone. The study results testify that, it is necessary to use *M.tuberculosis* isolated from the patients of the institution, to conduct disinfectant testing in TB institutions.

Keywords: tuberculosis, Mycobacterium tuberculosis, test-strains, disinfectants, disinfectant neutralizers.

In the adverse epidemiological situation with tuberculosis and mycobacterioses existing at the present time, the role of anti-epidemic strategies of a nonspecific character, practiced in medical-prophylactic institutions (MPI), becomes increasingly important, and decontamination of objects using various disinfectants is the crucial component of these strategies [6,7,5]. It is commonly known, that even provided the guidelines on disinfectant use are adhered to properly, effective disinfection will be achieved only if a test-microbe with resistance comparable to virulent hospital strains, has been used during the tryout of disinfection regimes [3,4].

In Russian Federation, "Mycobacterium B-5" strain is used as the test-microbe for determination of tuberculocidal activity of new disinfectants. Still, a possibility is never excluded that disinfection might prove to be inefficient, leading to the spread of tuberculosis and mycobacterioses causative organisms, especially in the in-patient tuberculosis clinics – the

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places, accumulating infected persons with the most active disease form who expectorate bacteria [8]. The most reasonable way out of this situation is to test the disinfectants in bacteriologic laboratory department of tuberculosis (TB) clinic, for tuberculocidal activity against mycobacterial cultures, isolated from the patients of the clinic.

The aim of the study was to determine the efficacy of recommended disinfectant use regimes against *M.tuberculosis* cultures isolated from the patients.

Materials and methods. This work deals with the chemical way of disinfection, i.e. disinfection using various chemical substances that destroy the causative organisms of infectious diseases. These substances include chlorine compounds (haloids), phenols, aldehydes, surfactants, gaseous disinfectants, etc.

We tried the disinfectant test procedure, intended for examining tuberculocidal properties of disinfectants accepted for use in TB institutions, and was designed by the Federal State Institution "Ural Research Institute of Phthisiopulmonology" of the Federal Agency for High-Tech Medical Care of the Russian Federation (Improved medical procedure: "The method of assessing the efficacy of disinfectants accepted for use in tuberculosis institutions", 2008).

The test procedure studied is to be incorporated then to accepted MPI practice, and is based on procedures adopted from a well-known method commonly used in disinfectant testing practice, which consists in submerging coarse calico test-objects contaminated with the test-microbes into a disinfectant.

The new features introduced to accepted national disinfectant test practice are: an experimentally approved use of mycobacterial isolates from patients as the test-microbes; adoption of ready-made Dey-Engley medium as a universal disinfectant neutralizer; and use of Lowenstein-Jensen medium in tubes instead of Petri dish.

Using this test procedure, we assessed tuberculocidal and mycobactericidal efficacy of various disinfectants accepted for use in TB institutions: 1. "Chloramine B"; 2. "Sulphochlorantine D"; 3. "Chlormisept-R"; 4. "Slavin"; 5. "Mirodez-univer"; 6. "Ecobriz"; 7. "Brilliant"; 8. "Aqua-chlor"; 9. "Alphadez".

The following test-strains were used to assess disinfectant efficacy: 1. Museum strain "Mycobacterium B-5"; 2. Museum strain M.bovis (strain 14, All-Russian Research Institute on Brucellosis and Tuberculosis in Animals); 3. Clinical strain M.tuberculosis № 255, resistant to

streptomycin at 10 mcg/mL MIC, to isoniazid at 1 mcg/mL MIC, to rifampicin at 40 mcg/mL, and to capreomycin at 30 mcg/mL; 4. Drug-susceptible clinical strain of *M.tuberculosis* № 258.

Results and discussion. Tuberculocidal efficacy of disinfectant use was assessed by the following reactions:

A. Presence of colony growth of the test-microbe both on test-object and on medium indicates that the disinfectant under test does not provide reliable tuberculocidal (mycobactericidal) effect in the given concentration and exposure time.

B. Absence of colony growth of the test-microbe neither on test-object, nor on medium indicates that the disinfectant's tuberculocidal and mycobactericidal properties are efficient enough to meet the requirements to disinfectants (ensure reduction of the seeding count of an object by 10⁵ CFU·sm⁻²), that allow them to be used in practice.

More than 500 control samples were collected and tested, to assess the efficacy of 9 disinfectants, belonging to the following groups of chemical compounds:

I. Chlorine compounds: 1. "Chloramine B" (in the concentration 0.5 %); 2. "Sulphochlorantine D" (1.0 %); 3. "Chlormisept-R" (0.2 %); 4. "Slavin" – (1.2 %); 5. "Brilliant" – (2.0 %); 6. "Aqua-chlor" (0.1 %).

II. Q.A.C. (quaternary ammonium compounds): 1. "Mirodez-univer" (1.0 %); 2. "Ecobriz" (2.0 %); 3. "Alphadez" (1.0 %).

Monitoring of viable bacterial cell numbers on the contaminated test-object was carried out at relevant phases of test procedure. Monitoring results are presented in *Table 1*.

Concentration of live mycobacteria on the test-object was calculated by the formula:

$$X = A \times 1000$$
, where

X – concentration of live mycobacteria on the test-object;

A – mean number of colony-forming units (CFU) across 5 tubes;

1000 – coefficient, resulting from the relation of 100 mL (total water volume inside a flask) to 0.1 mL (the suspension volume utilized for inoculation).

For example: Let " $Mycobacterium\ B-5$ " strain show the following growth counts: 1st sample $-122\ CFUs$, 2nd sample $-102\ CFUs$, 3rd sample $-120\ CFUs$, 4th sample $-92\ CFUs$, 5th sample $-105\ CFUs$, then:

mean number of CFUs across 5 tubes would equal:

$$A = (122+102+120+92+105) : 5 = 108$$



X = 108x1000 = 1080000, which corresponds to 1 mln. microbial bodies being present on the test-object.

From the *Table 1*, it is apparent that, according to calculations by formula, the number of viable bacterial cells on contaminated test-objects corresponds to 10⁶ microbial bodies per 1 mL.

Then, in order to monitor neutralizer efficacy and neutralization rate, a suspension method was utilized, which involves carrying-out of an experiment described in *Table 2*, showing main operations and their purpose.

The results of disinfectant efficacy assessment are presented in *Table 3*. All disinfectants tested, although they are widely accepted in health care practice, showed a bactericidal effect on "*Mycobacterium B-5*" alone, in 78% of cases. This supports the findings made by other authors before, who pointed at low resistance of "*Mycobacterium B-5*" strain to disinfectants, compared to museum and clinical strains [1].

Most Q.A.C.-based disinfectant solutions, even in the regimes recommended for accepted practice, failed to demonstrate 100% tuberculocidal or mycobacteriocidal effect on the following museum and clinical test-strains: "Mycobacterium B-5", M.bovis, multidrug-resistant M.tuberculosis No. 255, drug-susceptible M.tuberculosis No. 258.

Efficacy of chlorine disinfectants in recommended regimes was: 100% (i.e. no viable microbes present on test-object) – against "Mycobacterium B-5" test-strain, 50% – against M.bovis, 33% – against clinical multidrug-resistant M.tuberculosis No. 255 strain, and 67% – against clinical drug-susceptible M.tuberculosis No. 258 strain. Thus, on average, only 60.0% of the chlorine solutions tested had a destructive effect on causative organisms or tuberculosis.

Analysis of the study results showed that both museum and clinical test-strains of *M.bovis* and *M.tuberculosis* are more pertinent to real tuberculosis causative microorganisms in terms of resistance to disinfectants. The study findings support the feasibility of using a number of various test-strains instead of "*Mycobacterium B-5*" alone, in the development of national tuberculocidal disinfectant procedures.

The study results testify that, it is necessary to use *M.tuberculosis* isolated from the patients of the institution, to conduct disinfectant testing in TB institutions.

Conclusions:

- 1. All disinfectants accepted for use against tuberculosis infection belong to toxicity class 3 or 4, and should be applied in high concentrations and with longer exposure time.
- 2. On average, only 60.0% of the chlorine disinfectants solutions had a destructive effect on causative organisms of tuberculosis, isolated from patients.



3. Disinfectants must be adopted for use in TB institution strictly after susceptibility testing against mycobacteria, prevailing inside that institution.

Table 1

Monitoring of bacterial cell count on contaminated test-objects

No.	Museum and clinical test-	Growth rates of mycobacteria contaminating test-				
	strains of mycobacteria	objects, on solid nutrient medium (CFU*)				
	tuberculosis	Sample	Sample	Sample	Sample	Sample
		No. 1	No. 2	No. 3	No. 4	No. 5
1.	"Mycobacterium B-5"	122	102	120	92	105
2.	M. bovis (strain 14, All-Russian Research Institute on Brucellosis and Tuberculosis in Animals)	87	120	95	102	98
3.	M. tuberculosis No. 255, multidrug-resistant	95	98	103	108	96
4.	M. tuberculosis No. 258, drug-susceptible	75	93	132	103	102

^{*} CFU – colony-forming units

Table 2

Assignment of operations during experiment, checking the efficacy of neutralization of the residual disinfectant activity

Sample No.	Operation purpose	Operation procedure	Expected result
1.	Monitoring of the destructive activity of disinfectant	9 mL of test-strain (10 ³ CFU/mL) suspension in distilled water + 1 mL of disinfectant solution	Microbial growth must be absent



2.	Monitoring of the efficacy of disinfectant	9 mL of test-strain (10 ³ CFU/mL) suspension in	
	neutralization	neutralizer + 1 mL of	Approximately
		disinfectant solution	similar colony counts
3.	Monitoring of the absence	9 mL of test-strain (10 ³	in culture samples
	of antimicrobial activity	CFU/mL) suspension in	(0.1 mL each), on
	of neutralizer	neutralizer + 1 mL of	solid nutrient
		neutralizer	medium
4.	Reference-control of	9 mL of test-strain (10 ³	
	mycobacteria count	CFU/mL) suspension in	
		distilled water + 1 mL of	
		distilled water	

NOTE: 5 min after setting-up of the experiment, 0.1 mL of mixture from each 4 samples are harvested to at least 3 sloped tubes, containing nutrient medium, which are then incubated at 37°C. Reading of the results was done 5-7 days later.

Table 3

Assessment of disinfectant efficacy against mycobacterial test-strains

		Efficacy of disinfectants				
No.		in recommended regimes of application*				
	Disinfectants	Museum strains		Clinical strains		
		B-5	M. bovis	M. tuberculosis No. 258, drug-susceptible	M. tuberculosis No. 255, MDR	
1.	"Slavin"**	No growth	Growth	Growth	Growth	
2.	"Sulphochlorantine D"****	No growth	Growth	No growth	No growth	
3.	"Chlormisept-R"***	No growth	Growth	Growth	Growth	
4.	"Ecobriz"****	No growth	No growth	Growth	Growth	
5.	"Alphadez"***	Growth	Growth	Growth	Growth	
6.	"Chloramine B"****	No growth	No growth	No growth	Growth	
7.	"Mirodez-univer"***	Growth	No growth	No growth	Growth	
8.	"Brilliant"**	No growth	No growth	No growth	Growth	
9.	"Aqua-chlor"***	No growth	No growth	No growth	No growth	

Notes: * – in the disinfectant instructions for use, the regime is recommended for disinfection of linen, dishes, medical products, janitorial supplies etc.; ** - exposure 15 min; *** - exposure 30 min; **** - exposure 60 min; ***** - 120 min.



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The analysis of acute pneumonia morbidity in Republic Sakha (Yakutia) children

Morbidity statistic indexes and prophylactic medical examination of children with acute pneumonia in the Republic Sakha (Yakutia) was analyzed. In the group of children with acute pneumonia concomitant pathology, disease current features and treatment efficacy was studied.

Keywords: acute pneumonia, morbidity, children population, treatment efficacy.

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MORBIDITY OF POPULATION OF REPUBLIC OF SAKHA (YAKUTIA) BY PRIMARY CANCER OF LIVER

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The analysis of the primary cancer of liver morbidity (1658 cases) for 1996-2007 in Republic Sakha is conducted. A retrospective analysis allowed to mark more than 4th multiple exceeding of indexes of morbidity of primary cancer of liver of yakutian population by comparison to indexes on Russia. The exposed distinctive information on prevalence of morbidity in the different medical geographic areas of republic can be used for development of measures of prophylaxis of this pathology.

Keywords: primary liver cancer, prevalence, structure, dynamics, prognosis.

Introduction. Actuality of problem of primary liver cancer (PCL) in the conditions of Yakutia is conditioned by high-frequency of distribution of morbidity, unsatisfactory organization of prophylactic work, difficulties of active exposure of disease on the early stage. In Yakutia the study of different aspects of problem of this pathology was conducted it is not enough. Meantime the detailed study of features of distribution of primary cancer of liver with the estimation of possible etiologic factors and reasons of unsatisfactory organizational measures would allow to perfect methods of primary prophylaxis and diagnostics of PCL in Republic