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RESEARCH ARTICLE

The sensitivity of the strains of the pathogen *B. anthracis* and Anthrax-like *Bacilli* for Antibiotics

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An important test is the study of sensitivity to antibiotics in certain strains, which is created for the anti-anthrax drug as well as the direction of antibiotic therapy in the treatment of the disease. According to the order N 23 from 04.04.02, on the territory of Ukraine it is forbidden to use drugs which contain the following active substances: nitrofurans, including furazolidone, ronidazole, dapsone, chloramphenicol (chloramphenicol), dimetridazole, colchicine, chlorpromazine, chloroform, metronidazole, due to their harmful effects on the body, in particular mutagenic and teratogenic effects. That is why the introduction of antibiotic therapy to avoid the development of resistance to the pathogen *B. anthracis* is relevant. The research was conducted on the basis of the State Center for Innovative Biotechnology: *B. anthracoides* 67, *B. subtilis* 17, *B. cereus* 8035, *B. anthracis* Tsenkovsky II IBM 92 Z, *B. anthracis* K–79 Z, *B. anthracis* Stern 34F2, *B. anthracis* 55, *B. anthracis* SB, *B. anthracis* Tsenkovsky I. We determined the sensitivity to the antibiotic by the size of the diameter of the zones of growth retardation of the strain on nutrient agar. We considered the growth retardation of the strain up to 15 mm as a sign of low sensitivity to the antibiotic, a sign of regulatory sensitivity was considered a zone of 15–24 mm, the zone of more than 24 mm – a sign of high sensitivity of the strain to the antibiotic. In the absence of zones of growth retardation of the microorganism, the strain was insensitive to the antibiotic. According to the results of research it is established that benzylpenicillin does not affect the growth of strains *B. anthracoides* 67, *B. subtilis* 17, *B. cereus* 8035, *B. anthracis* Tsenkovsky II IBM 92 Z, *B. anthracis* 55, *B. anthracis* SB, *B. anthracis* Tsenkovsky I. Benzylpenicillin has a weak effect on strains of *B. anthracis* K–79 Z and *B. anthracis* Stern 34F2. Vaccine strains of *B. anthracis* K–79 Z, *B. anthracis* Stern 34F2, *B. anthracis* 55, *B. anthracis* SB showed high sensitivity to amoxiclav, tetracycline, norfloxacin. The vaccine strain of *B. anthracis* Stern 34F2 showed high sensitivity to furazalidone. We plan to continue the study of antibiotic susceptibility of *B. anthracis* strains in the future, in order to prevent antibiotic resistance in the treatment and prevention of the disease.

Keywords: *B. anthracis*; Sensitivity; Resistance; Antibiotics

Introduction

B. anthracis is an acute anthropozoonotic disease that primarily affects herbivorous mammals caused by *Bacillus anthracis* spores. The disease primarily affects domestic and wild herbivores. This disease is widespread throughout the planet and remains enzootic in many regions of Russia and the world, especially in sub-Saharan Africa, Asia and Central and South America. People are most often infected by infected animals or their products such as meat, animal skins, bones (Shadomy et al., 2016). *B. anthracis* disease is often associated with agriculture, the disease most often affects young and middle-aged people. Mortality is rare with appropriate therapy, but in the absence of treatment, the mortality rate can be up to 20% (Siddiqui, M. et al., 2012).

Resistance of pathogen spores to environmental factors, long-term preservation of biological qualities (more than a hundred years), the presence of lethal, fast-acting toxin created the conditions for the use of *B. anthracis* as a lethal weapon (Bengis & Frean, 2014, Ahmadi et al., 2015, Baillie, 2005). Anthrax spores are highly resistant to external influences and remain viable under the most adverse external conditions (Hugh-Jones & Blackburn, 2009). Studies of spore-forming bacteria have shown resistance to chemicals and high temperatures. We found that resistance to chemical disinfectants and temperature factors can vary both in different species of microorganisms and between strains of the same species (Melnikova & Panin, 1980).

There are 2,000 to 20,000 cases of Anthrax worldwide each year (Waits et al., 2018; Logvin et al., 2017). There is a need for a rapid and effective response to anthrax outbreaks, given the risk of anthropozoonotic infection. The main means of prevention and control of anthrax is the vaccination of animals and in case of infection – the use of antibiotics (*InstruktsIya pro zahodi z profIlaktiki ta borotbi z sibIrkoyu tvarin*) Instructions on measures to prevent and control anthrax in animals, 2000).

In Ukraine, spore vaccines from Sterne strains, K79Z, UA–07 are used for prophylactic and forced vaccinations of animals against anthrax (Skripnik & Kovalenko, 2011). Modern anthrax vaccines, made from spores of vaccine strains, are spore suspensions preserved with 30% glycerol with or without the addition of appropriate adjuvants. The reliability of such vaccines and the duration of post-vaccination immunity depends primarily on the quality of the anthrax strain used to make the vaccine. An important step in the study of such strains is to determine the sensitivity to antibiotics.

Recently, the problem of antibiotic resistance has become widespread. Irrational use of antibacterial drugs can lead to the emergence of antibiotic-resistant strains in naturally occurring disease (Cavallo et al., 2002). Determination of antibiotic susceptibility is one of the priority areas of research on anthrax and Anthrax-type bacilli strains. The antibiotic is used for treatment in the first hours after contact with Anthrax, despite reports of penicillin resistance in some strains (Mohammed et al., 2002). Therefore, the study of sensitivity to antibiotics is an important test for identifying strains for the design of against anthrax drugs and the direction of antibiotic therapy in the treatment of the disease.

Materials and Methods

The research was conducted on the basis of the State Center for Innovative Biotechnology. Strains of anthrax and anthrax-like bacilli from the collection of microorganisms of the State Center for Innovative Biotechnology were taken for experiments. To assess the sensitivity of the experimental strains were sown on nutrient agar in the amount of: B. *anthracoides* 67 – 5×106 CFU, *B. subtilis* 17 –3 × 10^6 CFU, *B. cereus* 8035 –3 × 10^6 CFU, *B. anthracis* Tsenkovsky II IBM 92 Z –2 × 10^6 CFU, *B. anthracis* K–79 Z – 3 × 10^6 CFU, *B. anthracis* Stern 34F2 –2 × 10^6 CFU, *B. anthracis* 55 –2 × 10^6 CFU, *B. anthracis* SB –5 × 10^6 CFU, B. *anthracis* Tsenkovsky I –3 × 10^6 CFU. We poured 25 cm^3 of medium into a cup with a diameter of 100 mm, the thickness of the agar in the cups was not less than 4 mm. We left the cups in the box at room temperature to solidify the agar. Before inoculation of bacterial masses, the plates were dried in a thermostat at a temperature of 35°C with the lid open for 10–20 min, to prevent the formation of condensate. For the study were used standard paper discs with a diameter of 7 mm, saturated with antibiotics, manufactured by Laboratories Pvt. Limited Mombay – 400086 India. Cups with seeding strains and applications of disks in the inverted state were cultured in a thermostat for 24 hours at a temperature of 37°C. After incubation, the plates were viewed against a dark background. The diameters of growth retardation of the studied strains of anthrax and anthrax-like bacilli were divided into: weakly sensitive to antibiotics – a zone of growth retardation of 1–15 mm; normatively sensitive - with a growth retardation of 15–24 mm; highly sensitive – with a growth retardation of more than 24 mm. That strain was considered insensitive to antibiotics when growth retardation in the area of application of the disc was absent.

Results and Discussion

The results for determining the sensitivity of vaccine strains of *B. anthracis* and anthrax-like bacilli are presented in Table 1.

Table 1. Susceptibility of anthrax strains and anthrax-like bacilli to antibiotics.

				The name of the strain								
№	**Antibiotics**	**disk**	**Notation on disk**	**B. *anthracoides* 67**	***B. subtilis* 17**	***B. cereus* 8035**	**B. *anthracis* Tsenkovsky II**	**B. *anthracis* K-79 Z**	**B. *anthracis Stern* 34F2**	**B. *anthracis* 55**	**B. *anthracis* SB**	**B. *anthracis* Tsenkovsky I**
1	Benzylpenicillin	100	P100	-	-	-	-	8	11	-	-	-
2	Amoxiclav	10	AC10	15	-	7	-	34	41	30	24	8
3	Streptomycin	10	S10	17	10	7	14	17	22	22	15	18
4	Erythromycin	10	E10	25	18	19	15	18	24	24	17	15
5	Ceftriaxone	10	Ci10	8	9	9	-	8	8	7	10	-
6	Tetracycline	10	T10	29	25	21	15	26	35	30	24	19
7	Norfloxacin	10	Nx10	29	17	18	22	25	30	27	25	19
8	Niroxalin	30	No30	24	18	15	11	15	20	14	18	7
9	Furazalidone	50	Fr50	21	15	12	18	24	30	22	20	12
10	Chloramphenicol	30	Лев	30	22	21	18	20	25	18	18	14

The results of the effect of antibacterial drugs on anthrax-like bacilli showed that B. *anthracoides* 67 is sensitive to most antibiotics taken in the experiment. Diameters of growth retardation are in the range from 8 to 20 mm. B. *anthracoides* was insensitive to benzylpenicillin and weakly sensitive to ceftriaxone. When comparing the zones of growth retardation, it was found that B. *anthracoides* is sensitive to 40% of antibiotics (amoxiclav, streptomycin, furazalidone, niroxalin) (Figure 1).

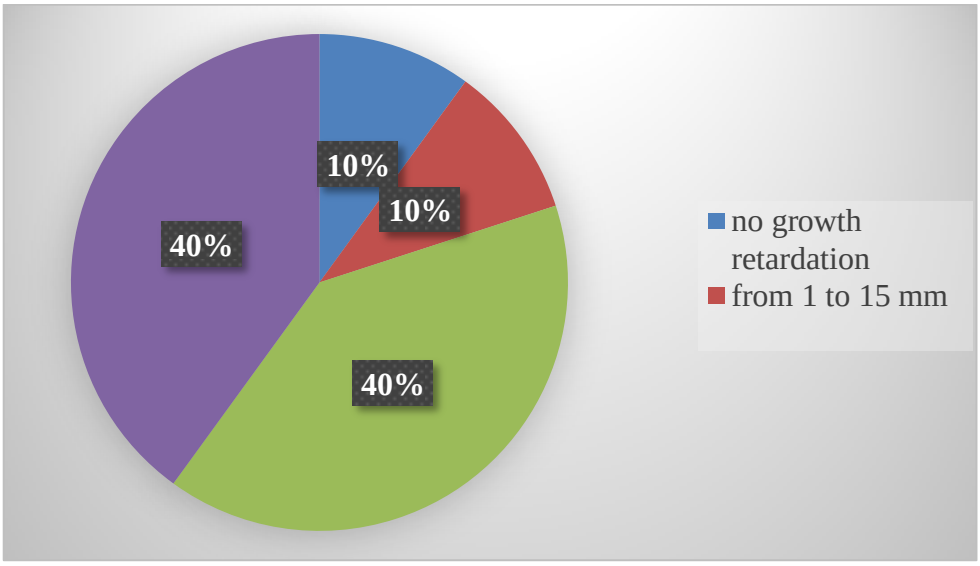

Figure 1. Antibiotic sensitivity of B. *anthrax*-like strain 67.

B. *anthracoides* 67 is highly sensitive to erythromycin, tetracycline, norfloxacin and chloramphenicol.

Strain *B. subtilis* 17 was insensitive to 20% of antibiotics (benzylpenicillin and amoxiclav) (Figure 2).

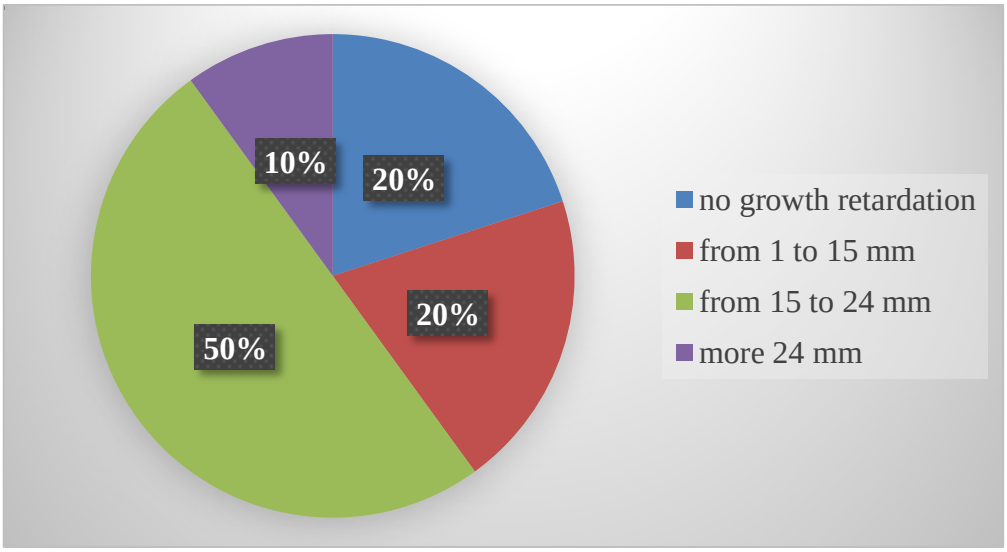

Figure 2. Antibiotic sensitivity of *B. Subtilis* 17.

Weak growth of *B. subtilis* 17 to streptomycin and ceftriaxone is established in the zones of growth retardation. The strain was sensitive to up to 50% of the studied antibiotics – niroxalin, tetracycline, erythromycin, streptomycin and chloramphenicol. *B. subtilis* 17 showed high sensitivity to tetracycline.

The sensitivity of *B. cereus* 8035 to antibacterial drugs is shown in Figure 3.

Strain *B. cereus* 8035 was insensitive to benzylpenicillin, as well as insensitive to 40% of the studied antibiotics (amoxiclav, streptomycin, ceftriaxone, furazalidone). *B. cereus* 8035 showed sensitivity to 50% of antibiotics (erythromycin, norfloxacin, tetracycline, chloramphenicol, niroxalin).

Analysis of the effect of antibacterial drugs on vaccine cultures of anthrax shows that the strain of *B. anthracis* Tsenkovsky II is insensitive to benzylpenicillin and amoxiclav and weakly sensitive to niroxalin and streptomycin (Figure 4).

The pathogenic strain of *B. anthracis* Tsenkovsky II showed sensitivity to 50% of the studied antibiotics (niroxalin, tetracycline, erythromycin, streptomycin, chloramphenicol). He was not sensitive to antibiotics such as benzylpenicillin, amoxiclav, ceftriaxone.

A study of the vaccine strain of *B. anthracis* K–79 Z showed that the strain showed weak sensitivity to benzylpecillin and ceftriaxone (Figure 5).

This strain is sensitive to 40% of the studied antibiotics – streptomycin, erythromycin, niroxalin, chloramphenicol, however, *B. anthracis* K–79 Z showed high sensitivity to amoxiclav, tetracycline, norfloxacin, furazalidone, which also accounted for 40% of the experimental drugs. Insensitivity to the studied antibiotics has not been established.

The antibiotic susceptibility study of *B. anthracis* strain Stern 34F2 showed that it is sensitive to all studied antibiotics (Figure 6). It is weakly sensitive to ceftriaxone and benzylpenicillin (20%). He has shown sensitivity to streptomycin and niroxalin (20%). High sensitivity in *B. anthracis* Stern 34F2 was detected to chloramphenicol, furazalidone, norfloxacin, erythromycin, tetracycline, amoxiclav (60%) (Figure 6).

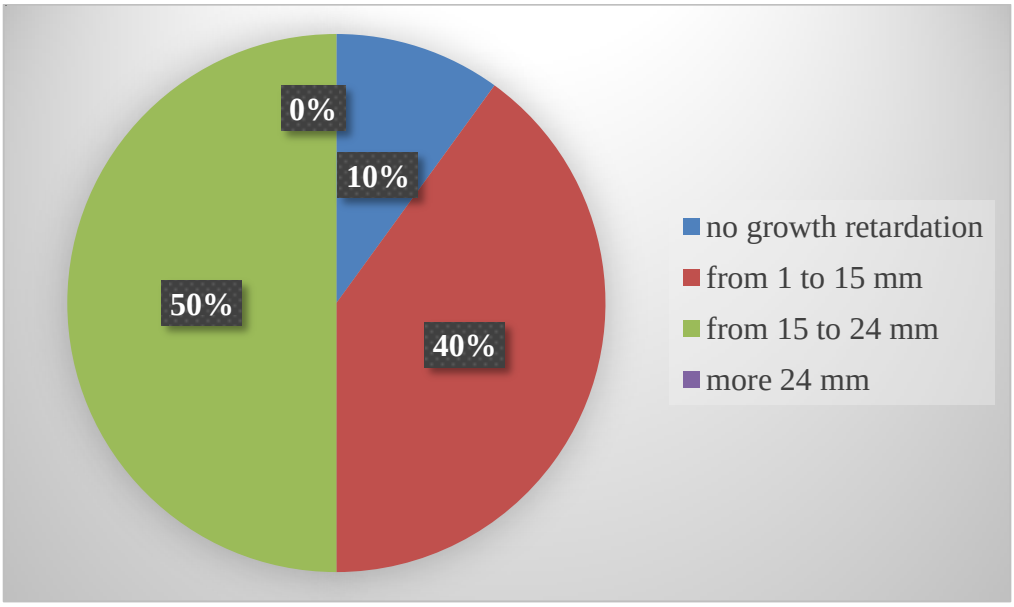

Figure 3. Antibiotic sensitivity of *B. cereus* 8035.

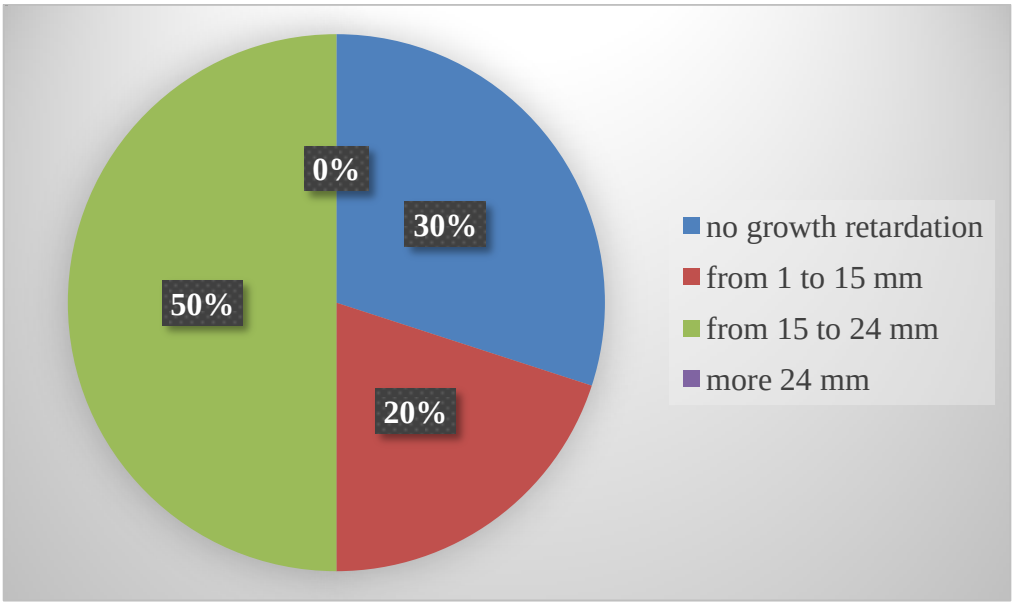

Figure 4. Antibiotic sensitivity of B. *anthracis* Tsenkovsky II.

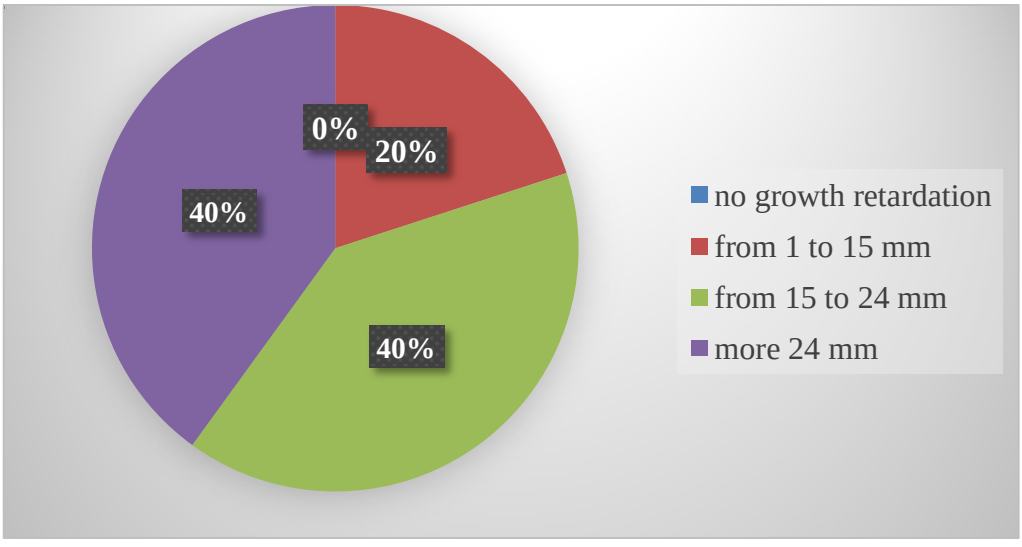

Figure 5. Antibiotic sensitivity of B. *anthracis* K–79 Z.

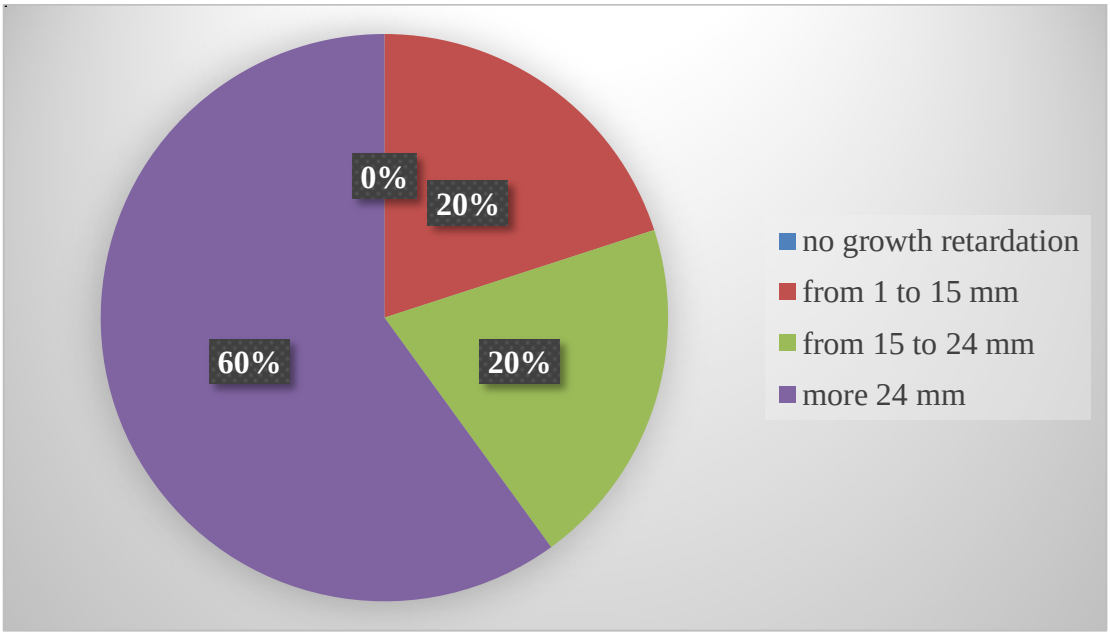

Figure 6. Antibiotic sensitivity of B. anthracis Stern 34F2.

A study of the strain *B. anthracis* 55 showed that the strain is not sensitive to benzylpenicillin (Figure 7). It is weakly sensitive to ceftriaxone and niroxalin and sensitive to streptomycin, furazalidone and chloramphenicol (30%). This strain showed high sensitivity to amoxiclav, erythromycin, tetracycline and norfloxacin (40%).

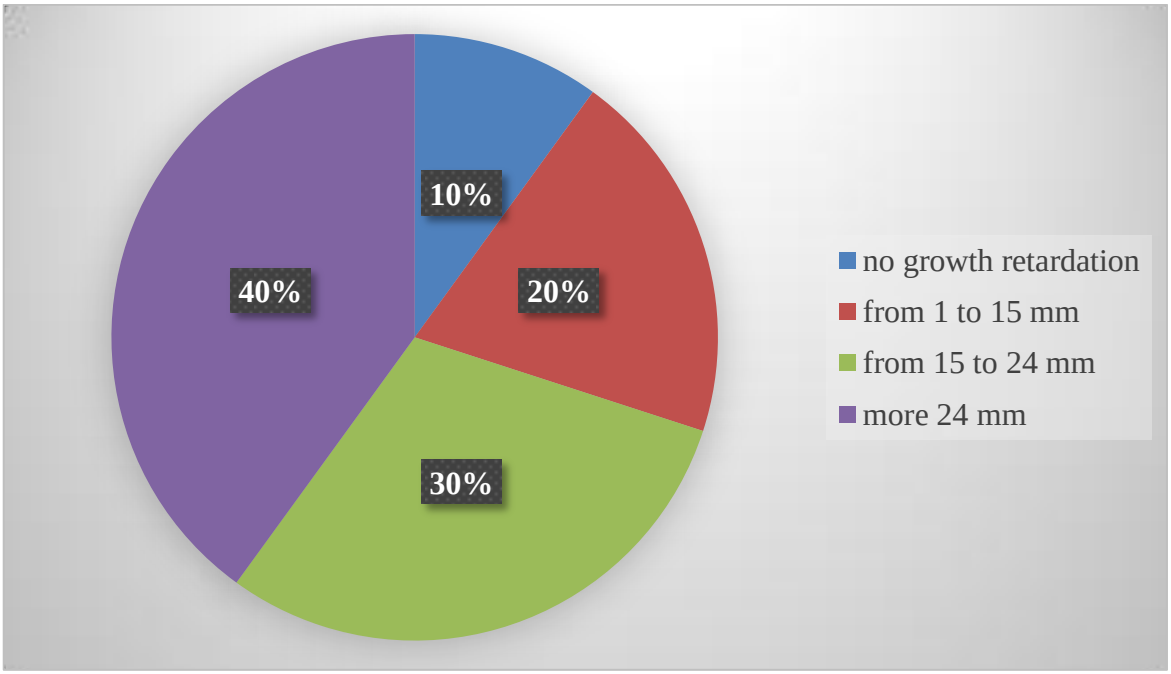

Figure 7. Antibiotic sensitivity of B. *anthracis* 55.

The vaccine strain of *B. anthracis* SB was insensitive to benzylpenicillin (Figure 8).

This strain showed low sensitivity to ceftriaxone. *B. anthracis* SB is sensitive to streptomycin, erythromycin, niroxalin and chloramphenicol (50%). Also up to 30% of antibiotics were highly sensitive – amoxiclav, tetracycline and norfloxacin.
According to the results of research, it was found that the strain of *B. anthracis* Tsenkovsky I was insensitive to 20% of antibiotics (benzylpenicillin and ceftriaxone) (Figure 9).

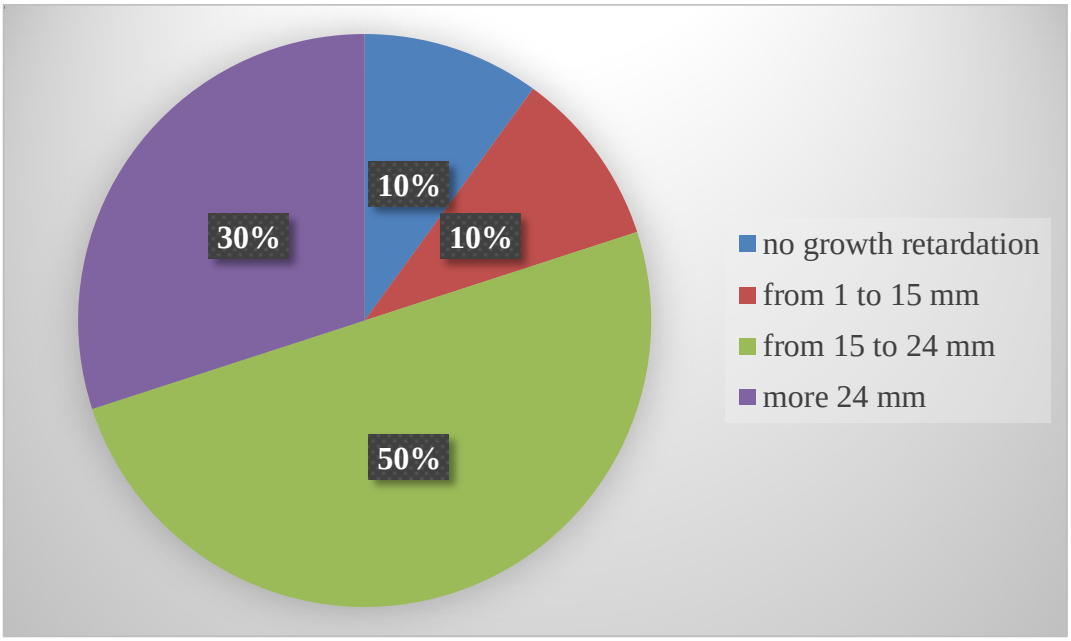

Figure 8. Antibiotic sensitivity of B. anthracis СБ.

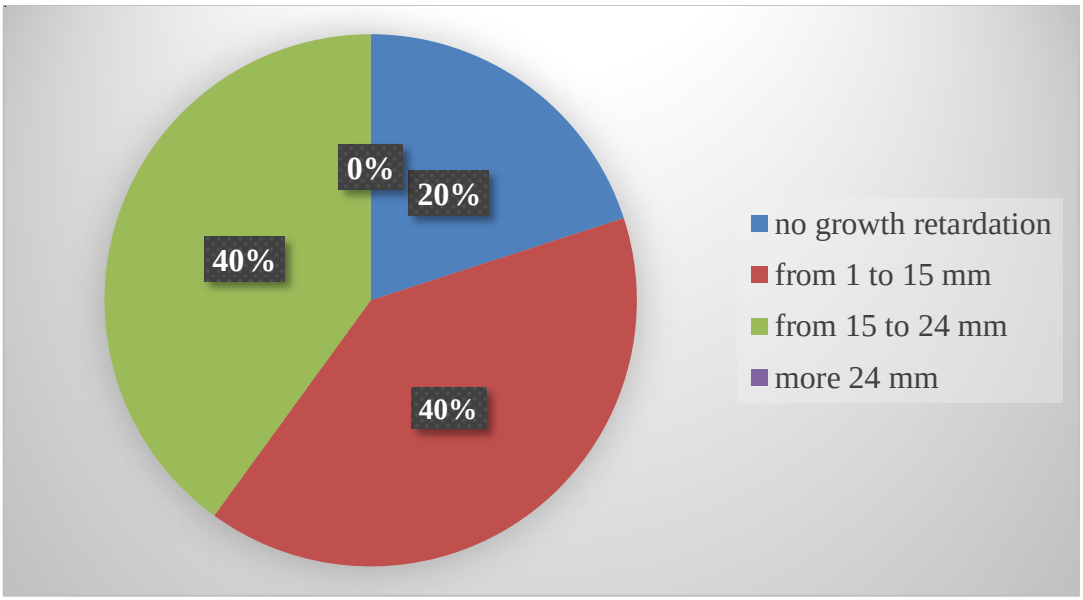

Figure 9. Antibiotic sensitivity of B. *anthracis* Tsenkovsky I.

B. anthracis Tsenkovsky I showed low sensitivity to chloramphenicol, furazalidone, niroxalin and amoxiclav and is sensitive to 40% of antibiotics: erythromycin, streptomycin, tetracycline and norfloxacin.

According to the order N 23 from 04.04.02, on the territory of Ukraine it is forbidden to use drugs which contain the following active substances: nitrofurans, including furazolidone, ronidazole, dapsone, chloramphenicol (chloramphenicol), dimetridazole, colchicine, chlorpromazine, chloroform, metronidazole, due to their harmful effects on the body, including mutagenic and teratogenic effects. A significant part of the experimental antibacterial drugs that had a positive effect on vaccine strains of anthrax and anthrax-like bacilli belong to the above list (chloramphenicol, furazolidone, norfloxacin). Their long-term use does not cause resistance in the experimental pathogen. It is this ban on their use that is the reason for our search for new effective antibiotics for anthrax.

Conclusion

According to the results of research it is established that benzylpenicillin does not affect the growth of experimental strains of anthrax-like bacilli and anthrax, namely: B. *anthracoides* 67, *B. subtilis* 17, *B. cereus* 8035, *B. anthracis* Tsenkovsky II IBM 92 Z, *B. anthracis* 55, *B. anthracis* SB, *B. anthracis* Tsenkovsky I. Benzylpenicillin has a weak effect on strains of *B. anthracis* K–79 Z and *B. anthracis* Stern 34F2. Vaccine strains of *B. anthracis* K–79 Z, *B. anthracis* Stern 34F2, *B. anthracis* 55, *B. anthracis* SB showed high sensitivity to amoxiclav, tetracycline, norfloxacin. The vaccine strain of *B. anthracis* Stern 34F2 showed high sensitivity to furazalidone. We are currently working on our prospects for further antibiotic susceptibility studies of anthrax strains, in order to prevent antibiotic resistance in treatment and prevention of the disease.

References

Shadomy, S., Idrissi, A. E., Raizman, E., Bruni, M., Palamara, E., Pittiglio, C., & Lubroth, J. (2016). Anthrax outbreaks: a warning for improved prevention, control and heightened awareness V.37. — september EMPRES-AnimalHealth@fao.org. Retrived from www.fao.org/ag/empres.html.

Siddiqui, M., Khan, M., Ahmed, S., Anwar, K., Akhtaruzzaman, S. M., Salam, M. (2012). Recent outbreak of cutaneous anthrax in Bangladesh: clinico-demographic profile and treatment outcome of cases attended at Rajshahi Medical College Hospital. BMC Research Notes, 28 Aug, 5:464. DOI: 10.1186/1756-0500-5-464.

Bengis, R. G., & Frean, J. (2014). Anthrax as an example of the One Health concept. Rev. sci. tech. Off. int. Epiz, 33 (2), 593–604. DOI: 10.20506 / rst.33.2.2309.

Ahmadi, A. H; Honari, H. & Minaei, M. E. (2015). Cloning, fusion, and expression of domain a-1 protective antigen (PA20) of Bacillus anthracis and N-terminal ipaD gene of Shigella in E. coli. Qom Univ. Med. Sci. J., 9: 20–29.

Baillie, L. W. J. (2005). Under the microscope Bacillus anthracis, a story of nature subverted by man. Letters in Applied Microbiology, 41, 227–229. doi:10.1111/j.1472-765X.2005.01786.

Hugh-Jones, M. & Blackburn J. (2009). The ecology of Bacillus anthracis. Mol Asp Med.; 30:356–67. DOI: 10.1016 / j.mam.2009.08.003.

Melnikova, G. N., & Panin, O. G. (1980). Sravnitelnaya ustoychivost muzeynyih shtammov spor batsill k deystviyu nekotoryih sterilizuyuschih i dezin-fitsiruyuschih agentov. Problemyi dezinfektsii i sterilizatsii: Sb. nauch. trudov. M. 46–50. (in Russian).

Waits, A., Emelyanova, A., Oksanen, A., Abass, K., & Rautio, A. (2018). Human infectious diseases and the changing climate in the Arctic. Environ Int. Dec;121(Pt 1):703-713. DOI:10.1016 / j.envint.2018.09.042.

Logvin, F. V., Kondratenko, T. A., & Vodyanitskya, S. Y. (2017). Anthrax in the world, CIS and Russian Federation (literature review). Med Herald South Russia. 8(3).17–22. https://doi.org/10.21886/2219-8075-2017-8-3-17-22.

InstruktsIya pro zahodi z profIlaktiki ta borotbi z sibIrkoyu tvarin (2000). Retrived from https://zakon.rada.gov.ua/laws/show/z0136-00 (in Ukrainian).

Skripnik, V. G., Kovalenko, V. (2011). Sibirskaja jazva. The Ukrainian farmer. Retrived from ttp://www.agrotimes.net/magazines. (in Russian).

Cavallo, J.-D., Ramisse, F. Girardet, M., Vaissaire, J., Mock, M., & Hernandez, E. (2002). Antibiotic Susceptibilities of 96 Isolates of Bacillus anthracis Isolated in France between 1994 and 2000. Antimicrob Agents and Chemother.Vol. 46 (7), 2307–2309. DOI: 10.1128/AAC.46.7.2307-2309.2002.

Mohammed, M. J., Marston, C. K., Popovic, T., Weyant, R. S., & Tenover, F. C. (2002). Antimicrobial susceptibility testing of Bacillus anthracis: Comparison of results obtained by using the National Committee for Clinical Laboratory Standard broth microdilution reference and test agar gradient diffusion method. Journal of Clinical Microbiology, 40, 1902–1907. DOI:10.1128 / jcm.40.6.1902-1907.2002.

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