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SUSCEPTIBILITY OF BACILLUS SPECIES TO ANTIMICROBIAL AGENTS *IN VITRO*

The study was conducted to determine the prevalence and antibiotic susceptibility pattern of Bacillus species. The primary purpose of the study was to determine the susceptibilities of these species to a set of antibiotics with the greatest guidance value to veterinarians encountering anthrax, B. cereus, and possibly other wound infections. The generation of comparative susceptibility and resistance data on the B. cereus group was the secondary aim of the work.

It was carried out the investigation of antibacterial action and detected the minimal inhibiting concentration of antimicrobial drugs towards museum strains of Bacillus species.

Keywords: *antimicrobial action, microorganisms, Bacillus species, Bacillus anthracis*

Introduction. The low recognition of the ability of *Bacillus* species other than *Bacillus anthracis* to cause infections and the increasing rarity of anthrax in industrialized, developed countries as a result of effective control programs over the past half century and the high susceptibility of *B. anthracis* to penicillin coupled with the extreme rarity of reports of penicillin resistance lowering the interest to this infections until the very last time. In developing countries where anthrax is endemic, penicillin has always been the drug of choice because of its reliability, low cost, and ready availability. Concerns about bioaggression in 2001 resulted in some examination of the effectiveness of more modern antimicrobials both in vitro [1–3] and in vivo in animal models [4–6]. The «anthrax letter» events in the United States further stimulated interest in antimicrobial therapy for anthrax with *in vitro* susceptibility tests being carried out [4, 5–7].

The success of antibiotic therapy of animals infection diseases and control of epizootic hot spots along with preventive vacination is the main approaches to infectious diseases control and survailance.

According to global trends steadily increasing antimicrobial resistance of major respiratory pathogens as *Streptococcus pneumoniae* and *Haemophilus*

influenzae to penicillin, macrolides and tetracycline, but there was only few fundamental monitoring studies in Ukraine onto investigation of dinamics appearing resistant microorganisms to antibacterial agents, especially spatial-temporial data analysis of different geographical areas. There is the necessity to focus not only on geographical proximity, but also on historical tradition of using some antibiotic drugs in antiinfectious therapy [8–11].

This situation requires a search for new antimicrobial agents which have properties to decrease the formation of antimicrobial resistance. However, antibacterial drugs effective applying is limited by speed of microbial resistance manifestation to the most of modern antimicrobial drugs [3, 4, 9].

The emergence of antibiotic resistance is the urgent problem worldwide. Current antibiotics target a small set of proteins essential for bacterial survival. As a result, antibiotic resistant strains are subjected to a strong positive selection pressure [3]. Inappropriate and excessive use of antibiotics have contributed to the emergence of pathogens that are highly resistant to most currently available antibiotics.

The goal of the work. The study was conducted to determine prevalence and antibiotic susceptibility pattern of *Bacillus* species. The primary purpose of the study was to determine the susceptibilities of these species to a set of antibiotics selected to have the greatest guidance value to veterinarians encountering Anthrax, *B. cereus*, and possibly other wound infections. The generation of comparative susceptibility and resistance data on the *B. cereus* group was the secondary aim of the work.

Materials and methods of research. ***Bacterial strains.*** A total of 4 strains of *Bacillus* species were included in the study. All strains were identified by standard microbiological procedures. *Staphylococcus aureus* ATCC 29213 was also used in our study as quality control strain. The results of the broth microdilution method for the strain were within the expected ranges.

Broth microdilution reference method. Prior to testing, each isolate was subcultured on Brain Heart Infusion (BHI) plates that were incubated at 35°C overnight. The susceptibility of each bacterial culture to nine antimicrobial agents was tested by the broth microdilution reference method with 96-well microtiter panels prepared in laboratory. Growth from a BHI agar plate incubated at 35°C for 16h was suspended in 5 ml of Mueller-Hinton broth to the turbidity of a 0.5 McFarland standard. Two milliliters of this suspension was transferred to 38 ml of sterile water. The suspension was washed 5 times and then poured into a disposable inoculums tray. It delivers approximately 10 µl into a final volume of 100 µl/well. The broth microdilution panels were stacked no more than three high and placed into a self-sealing plastic bag in ambient air at 35°C for 16 to 24 h. The final inoculum was approximately 3×10^4 CFU/ml, as determined from the colony counts for the growth control well.

Antimicrobial susceptibility testing was performed on Muller-Hinton(MH) agar by disc-diffusion method against the following antibiotics: amoxicillin with clavulanic acid, oxytetracicline, ceftriaxon, ciprofloxacin, gentamicin, penicillin, sulfamethoxazole with trimethoprim according to laboratory standard guidelines.

Antimicrobial disc susceptibility testing was performed to the following antimicrobial agents using the standard disc diffusion method. Susceptibility to penicillin was determined in order to differentiate *B. anthracis* from *B. cereus* (Quinn et al., 1994). Depending on the zone of inhibition results were interpreted as susceptible, intermediary susceptible or resistant.

In the study it was used *Bacillus anthracis* (strain UA 07), *Bacillus cereus* (strain ATCC 10702), *Bacillus subtilis* (strain 1228) and *Bacillus mycoides* from the collection of The Institute of Veterinary Medicine of the NAAS.

Results of research and discussion. Differences between *B. anthracis* and the other species were as expected for penicillin and amoxicillin-clavulanic acid. *B. cereus* and *B. subtilis* that were resistant to doxycicline was also to be expected. Apparent species differences between *B. cereus*, *B. subtilis*, and *B. mycoides* were detected with doxycicline. *B. cereus* has long been associated with both food-borne pathogens and nongastrointestinal infections. The latter infections are usually, but not always, opportunistic and are sometimes severe or life threatening. The involvement of *B. thuringiensis* in infections is rare but has occurred, while *B. mycoides* appears to be totally nonpathogenic.

According to the results using the disc diffusion method, all strains were susceptible to penicillin (Tab. 1). Resistance to sulfamethoxazole with trimethoprim was determined in all strains. These results support the claim that sulfamethoxazole with trimethoprim should not be used in anthrax prophylaxis or treatment in humans (Esel et al., 2003). The minimum inhibitory concentration (MIC) of ciprofloxacin for *B. anthracis* strain UA 07 by method of serial dilutions was 0.25±0.01 mg/ml. The MIC of ciprofloxacin for *B. cereus* strain ATCC 10702 by method of serial dilutions was 0.35±0.01 mg/ml. The MIC of ciprofloxacin for *Bacillus subtilis* by method of serial dilutions was 0.3±0.01mg/ml.

In a comparison of MIC methods, all tested *Bacillus* species strains all were susceptible to ciprofloxacin and, with some variation between methods to doxycycline. The results for the antimicrobial MIC testing are indicated in along with the MIC ranges for each antibiotic and the breakpoint values (Tab.1). All the strains were resistant to streptomycin (MIC >8.0 mg/liter).

The results of this study show that *in vitro* increases in MIC for *B. anthracis* occur with various antibacterials belonging to different classes. Resistance within an antibiotic class may vary among the different class members.

Since treatment of anthrax is rather prolonged, continuous surveillance of susceptibility of *B. anthracis*, even if the original isolates are antibiotic susceptible, needs to be incorporated in public health measures of anthrax outbreak.

Table 1

Antimicrobial susceptibility investigation results for *Bacillus* species

Antibiotics	Species	MIC (mg/ml)		
		Range	50%	90%
Amoxicillin-clavulanic acid	*B. anthracis*	0.016-0.5	0.032	0.047
	B. cereus	0.5-64	8	12
	B. subtilis	4-96	12	24
	B.mycoides	8-24	8	24
	S. aureus	0.5(0.25-1)		
Azithromycin	*B. anthracis*	1-12	3	6
	B. cereus	0.094-6	0.38	3
	B. subtilis	0.094-3	0.19	3
	B.mycoides	0.19-0.38	0.19	0.38
	S. aureus	2.5 (1.5-4)		
Ceftrtiaxone	*B. anthracis*	3->32	>32	>32
	B. cereus	0.1->32	>32	>32
	B. subtilis	>32	>32	>32
	B.mycoides	>32	>32	>32
	S. aureus	1.5(1.5-2)		
Ciprofloxacin	*B. anthracis*	0.032-0.094	0.064	0.094
	B. cereus	0.047-0.5	0.19	0.25
	B. subtilis	0.094-0.19	0.125	0.19
	B.mycoides	0.125-0.25	0.125	0.25
	S. aureus	0.5(0.25-0.5)		
Gentamicin	*B. anthracis*	0.064-0.5	0.25	0.38
	B. cereus	0.094-0.75	0.38	0.75
	B. subtilis	0.047-0.5	0.19	0.5
	B.mycoides	0.19-0.38	0.25	0.25
	S. aureus	0.8(0.5-1.5)		
Penicillin	*B. anthracis*	<0.016-≥32	<0.016	0.023
	B. cereus	0.012->32	>32	>32
	B. subtilis	>32	>32	>32
	B.mycoides	>32	>32	>32
	S. aureus	0.35(0.25-0.38)		
Oxytetracycline	*B. anthracis*	0.016-0.094	0.023	0.032
	B. cereus	0.05-0.32	1	6
	B. subtilis	0.5-24	2	6
	B.mycoides	0.125-2	0.5	2
	S. aureus	0.17(0.094-0.38)		
Sulfamethoxazole with trimethoprim	*B. anthracis*	0.016-0.094	>32	>32
	B. cereus	0.05-0.32	>32	>32
	B. subtilis	0.5-24	>32	>32
	B.mycoides	0.125-2	>32	>32
	S. aureus	0.4(0.35-0.48)		

Disk diffusion susceptibility testing of *B. cereus* proved that it was resistant to penicillin, oxytetracicline, and cephalosporins. However, penicillin susceptibility may be a better indicator of *in vivo* effectiveness for P-lactamase producers such as *Bacillus spp*. In contrast, according to literature data, many non *B. cereus* strains were susceptible to penicillin, cephalosporins, and trimethoprim-sulfamethoxazole.

Using standard breakpoints for disk diffusion susceptibility testing proved highly accurate in predicting results obtained by microdilution. All *Bacillus* strains classified as susceptible by disk diffusion susceptibility testing were in fact susceptible by microdilution testing and strains classified as intermediate were either susceptible or intermediate susceptible by microdilution testing.

All tested strains showed reduced susceptibility to ceftriaxone (MIC ≥32 μg/ml), oxytetracycline (MIC ≤0.05 μg/ml), and gentamicine (MIC ≤0.38–0.75 μg/ml) by use of breakpoints for staphylococci. High susceptibility was shown by all tested microorganisms to ciprofloxacin (MIC 0.064–0.125 μg/ml), azitromicin (MIC 0.19–6 μg/ml), amoxicillin-clavulanic acid (MIC 0.032–12 μg/ml). *B.anthracis* strain tested shown high level of susceptibility to amoxicillin-clavulanic acid, ciprofloxacin, penicillin, oxytetraciclin (MIC ≤0.06μg/ml); the moderate level of susceptibility was detected toward azytromycin (MIC ≤3μg/ml), gentamicin (MIC ≤0.25μg/ml), and was resistant to ceftriaxone. Unlike the *B. anthracis*, all other tested strains were resistant to penicillin and amoxicillin with clavulamic acid (MIC ≥8 μg/ml).

Conclusions and prospects for futher research. Based on our *in vitro* data, the drug of choice for *Bacillus* infections proved to be penicillin and amoxicillin with clavulanic acid. It was also detected highly active drugs with bactericidal action: gentamicin, ciprofloxacin and azytromicin. Most *Bacillus* strains were resistant to broad-spectrum cephalosporins and amoxicillin-clavulanate. These agents should be avoided in the empiric coverage of animals and humans, especially if immunocompromised, who have had a grampositive aerobic bacillus isolated until susceptibility testing is available.

Whether single-drug therapy is adequate or combination therapy is advantageous, especially with an aminoglycoside, should be investigated *in vitro* and in animal models.

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IN VITRO ЧУВСТВИТЕЛЬНОСТЬ МИКРООРГАНИЗМОВ РОДА BACILLUS К АНТИМИКРОБНЫМ ВЕЩЕСТВАМ / Бабкина М.М., Тарасов О.А., Нычик С.А., Сапейко Л.П., Терещенко С.Н., Гудзь Н.В., Гайдей А.С.

Исследование было проведено с целью определения распространенности и особенностей чувствительности к антибиотикам бактерий рода Bacillus. Основная цель исследования состояла в определении чувствительности этих видов бактерий к ряду антибиотиков, рекомендованные для применения ветеринарным врачам, работающих с возбудителем сибирской язвы, B. cereus и другими возможными раневыми инфекциями.

Второй целью работы было определение сравнительной чувствительности и резистентности бактерий группы Cereus.

Исследовано антибактериальное действие и выявление минимальной ингибирующей концентрации антимикробных препаратов в отношении музейных штаммов видов Bacillus.

Ключевые слова: *антимикробная чувствительность, микроорганизмы рода Bacillus, Bacillus anthracis, антимикробные вещества.*

IN VITRO ЧУТЛИВІСТЬ МІКРООРГАНІЗМІВ РОДУ BACILLUS ДО АНТИМІКРОБНИХ РЕЧОВИН / Бабкіна М.М., Тарасов О.А., Ничик С.А., Сапейко В.П., Терещенко С.М., Гудзь Н.В., Гайдей О.С.

Досліджено антимікробну активність антибіотиків (амоксіцилін-клавулонової кислоти, азітроміцину, цефтриаксону, ципрофлоксацину, гентаміцину, пеніциліну, оксітетрацикліну сульфометоксазолу з триметопримом) по відношенню до 4 музейних штамів роду Bacillus (B. anthracis UA 07, B. cereus ATCC 10702, B. subtilis 1228, B.mycoides). Проведено дослідження антибіотичної активності та визначення мінімальних інгибуючих концентрацій по відношенню до цих штамів Bacillus. Мінімальна інгібуюча концентрація визначалась методом серійних розведень та диско-дифузійним методом. Встановлено достатню бактерицидну активність антибіотиків та відсутність проявів резистентності у всіх досліджених музейних культур Bacillus.

Усі досліджувані штами показали знижену чутливість до цефтриаксону (МІК ≥32 мкг/мл), окситетрацикліну (МІК ≤0,05 мкг/мл) та гентаміцину (МІК ≤0,38–0,75 мкг/мл). Високу чутливість до ципрофлоксацину (МІК 0,064–0,125 мкг/мл), азітроміцину (МІК 0,19–6 мкг/мл) та амоксициліну-клавуланової кислоти (МІК 0,032–12 мкг/мл) показали всі тестовані мікроорганізми. Досліджений штам B. anthracis показав високий рівень чутливості до амоксициліну-клавуланової кислоти, ципрофлоксацину, пеніциліну,

оксітетрацикліну (МІК ≤0,06 мкг/мл). Помірний рівень чутливості був виявлений до азітроміцину (МІК ≤3мкг/мл), гентаміцин (МІК ≤0,25 мкг/мл). На відміну від B. anthracis усі інші випробувані штами були стійкими до пеніциліну та амоксициліну з клавулановою кислотою (МІК ≥8 мкг/мл).

Ключові слова: *антимікробна чутливість, мікроорганізми роду Bacillus, Bacillus anthracis, антимікробні речовини.*

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THE ROLE OF THE FAT IN LABORATORY DIAGNOSIS OF RABIES

The article presents analysis of accompanying notes for the specimens of pathological materials from animals diagnosed rabies and other reporting documentation. It was proved the importance of using fluorescent antibody test for laboratory diagnosis of rabies and shown the advantages and disadvantages of this method.

Keywords: *rabies, laboratory diagnosis, FAT.*

Introduction. Rabies has a great importance among infectious diseases. Sensitivity regard this diseases of all domestic and wild animals species and extreme danger to humans determine its social and economic importance and focus attention of veterinary, medical science and practice [1].

An important link in the control of rabies is laboratory diagnosis, because in veterinary laboratories of Ukraine about 1500 cases of rabies in animals confirmed annually [2].

Rabies is a major zoonosis for which diagnostic techniques have been standardized internationally [2]. So, **the goal of the work** was determination of role of the FAT for laboratory diagnosis of rabies in Ukraine and in foreign countries.

Materials and methods of research. In this work we used laboratory research expertise, reports of regional departments of veterinary medicine of the State Scientific Research Institute of Laboratory Diagnostic and Veterinary Sanitary Expertise of the State Veterinary and Phytosanitary Servise of Ukraine. Also we used the accompanying notes to the pathological material of animals diagnosed with rabies.

The results of research and discussion. At present, the rabies diagnosis in animals is based on complex epizootic, clinical, pathological and laboratory studies. But, as there are neither gross pathognomonic lesions nor specific and