STUDY OF SUSCEPTIBILITY OF ISOLATES ISOLATED FROM DISEASED ANIMALS TO ANTIBIOTIC PREPARATIONS COMPARED TO TEST MICROORGANISMS

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The paper shows the results of the study of the museum test cultures of microorganisms and isolates isolated from diseased animals sensitivity to six groups of antibiotic preparations by disk-diffusion method. It was found that the efficiency of their action to the isolates significantly less compared to the museum strains due to the formation of resistant microorganisms.

Keywords: *antibiotic preparations, strains, isolates, resistance.*

Today multidrug-resistant bacteria strains are a major concern for public health and animal health as well. The acquisition of antimicrobial resistance is a complex issue, which is related to the ability of bacteria to adapt quickly to changing environmental conditions. Resistance is a tool that allows the bacteria to survive and develop in response to adverse conditions [5–6].

Penetrating into the body of animals, bacteria can cause infectious diseases as single-agent infection or mixed infection, including common to animals and humans [7, 9–10]. Effectiveness of the therapy requires constant replacement of one antibiotic preparation to other, sometimes more expensive and toxic. In terms of animal husbandry complexes with a high concentration of livestock it is observed rapid formation of antibiotic-resistant strains of bacterial diseases that can complicate the selection of an optimal treatment regimen [1–4, 8].

The purpose of the study is to determine the sensitivity of the test microorganisms and pathogenic field isolates isolated from diseased pigs, to the most common antibiotic drugs.

Materials and research methods. We used strains of bacteria that are stored and maintained at the Institute of Veterinary Medicine, as well as a number of field isolates from diseased pigs (tabl. 1).

Cultivation of microorganisms was carried out on MPA, MIB, Sabouraud's medium, thioglycollate medium, which were prepared according to the instructions or generally accepted compositions and methods. Sterilization was conducted by autoclaving at a temperature 100-118°C within 30-60 minutes.

Table 1

Cultures of microorganisms used in the study

Item No.	Test museum cultures	Item No.	Field isolates
1.	*Micrococcus flavus* ATCC10240	1.	*E. coli*
2.	*Micrococcus luteus* ATCC 9341	2.	*Streptococcus zooepidemicus*
3.	*Bacillus subtilis* ATCC6633	3.	*Staphilococcus aureus*
4.	*Bacillus cereus* ATCC 11778	4.	*Klebsiella spp*
5.	*Staphylococcus aureus* P209	5.	*Pasterella multocida*
6.	*Erysipelothrix rhusiopathiae* VR-2		
7.	*E. coli* 1257		

In the experiment we used antimicrobials of cephalosporins, fluoroquinolones, aminoglycosides, penicillins, macrolides, tetracyclines and macrolides groups. To assess their activity we used disc diffuse method (DDM)

To arm the DDM it was used standardized discs that were applied with the most common antibiotic preparations. This method is based on their ability to diffuse from the paper discs into the medium and inhibit the growth of microorganisms inoculated in the depth or on the surface of the agar. We focused on a uniform layer agar in plates. In our experiments, it was 4,0±0,5 мм (20 см^3).

Suspension of microorganisms were used to inoculate a sterile saline solution equivalent to 0.5 units by optical McFarland standard (concentration of microorganisms was approximately 106 CFU / cm^3).

The 1 cm^3 of inoculum was applied to the surface of the nutrient medium in plates and evenly distributed over its entire surface.

Plates were dried in an incubator at 36 ± 1°C 30 minutes.

Discs with antiseptic substances were put on the surface of the medium using microtweezers, keeping 20 mm distance between the discs and the edge of the cup.

That is, no more than six disks with various antibiotic substances were applied in one plate. The plates were incubated at 36 ± 1°C for 24 hours.

Recording of growth inhibition zones (GIZ) of test microorganisms was performed using calipers. When determining the microorganisms GIZ only the zone of complete lack of visible growth were taken into account.

Results and discussion. *Micrococcus* bacteria showed high sensitivity to cephalosporins and amoxicillins antimicrobials – GIZ was 50 mm. The least effective were preparations of aminoglycosides (tabl. 2).

Bacillus culture showed different results. *B. subtilis* was the most sensitive to gentamicin – GIZ was 36 ± 1.0 mm, and fluoroquinolones – 30–32 mm. *B. cereus* showed moderate sensitivity to some antibiotic preparations, among which cephalosporin preparations were the most effective – GIZ 20–25 mm.

Well pronounced effect on the *Erysipelothrix rhusiopathiae* pathogen showed preparations of penicillin, cephalosporins and fluoroquinolones – GIZ ranged between 39–48 mm. Particularly noteworthy two preparations which showed the strongest effect which are amoxicillin (48 ± 1.2 mm) and cephalexin (41 ± 0.9 mm). The rest of the preparations were poorly or not effective at all.

St. aureus was sensitive to all preparations except erythromycin. The pathogen showed highest sensitivity to cephalosporins: cephalexin and cefazolin (GIZ was 27 mm), enrofloxacin (26 ± 0.5 mm) and gentamicin (25 ± 0.5 mm).

E. coli test culture showed sensitivity to fluoroquinolones (24–27 mm) and ceftriaxone (22 ± 0.4 mm). It was observed poorly effect of aminoglycoside and tetracyclines to this pathogen – GIZ ranged from 12 to 19 mm, and it was completely insensitive to the rest of preparations.

Klebsiella spp. pathogen showed the highest sensitivity to almost all antimicrobials of all the studied isolates. It showed the highest sensitive to ciprofloxacin – GIZ was 33 ± 1.0 mm and ceftriaxone – 28 ± 0.5 mm.

The rest of the isolates showed selective and significantly less sensitiveness to all preparations. Thus, *E. coli* isolate showed the highest sensitivity to cefazolin, the GIZ was 25 ± 0.4 mm, ceftriaxone and gentamicin – 20–21 mm.

St. zooepidemicus had the highest sensitivity to amoxicillin and erythromycin, GIZ were 24 ± 0,3 and 21 ± 0,5 mm respectively.

The most active antimicrobials to *P.multocida* pathogen were cephalexin, GIZ was 23 ± 0.4 mm, amoxicillin, and enrofloxacin, GIZ was 22 mm in both last cases.

St. aureus pathogen isolate showed the highest sensitivity to two preparations – cefazolin and enrofloxacin, which GIZ was 25 ± 0.3 and 22 ± 0.8 mm respectively.

CONCLUSIONS

The results of the studies found a difference of sensitivity of test cultures of microorganisms and isolates isolated from diseased animals to antimicrobial agents of different pharmacological groups. Field isolates in contrast to the test cultures of microorganisms of relevant groups showed sensitivity only to cephalosporins and fluoroquinolones antibiotic preparations. At the same time it was observed selective sensitivity to some preparations within the same group.

This difference of sensitivity of test cultures and isolates can be explained by the development of resistant strains of pathogens due to the widespread use of antibiotics in livestock farms.

We recommend rational approach to antibiotic therapy using different groups of antibiotic preparations in various stages of animals growing to reduce the risk of rapid formation of resistant strains of pathogens.

Table 2

The sensitivity of microorganisms to antibiotic preparations

Antibiotic preparation	Diameter of the microorganism growth inhibition zones, (mm)											
	Test museum culture							Isolate				
	Micrococcus flavus	Micrococcus luteus	Bacillus subtilis	Bacillus cereus	Erysipelothrix rhusiopathiae	Staphylococcus aureus 3209	E. coli 1257	E. coli	Streptococcus zooepidemicus	Klebsiella spp	Pasteurella multocida	Staphylococcus aureus
Cefalexin	50±2.0	50±2.0	28±1.0	25±0.6	41±0.9	27±1.0	—	—	—	20±0.2	23±0.4	11±0.5
Ceftriaxone	55±2.0	55±2.0	14±0.3	20±1.0	38±0.1	20±0.3	22±0.4	21±0.5	—	28±0.5	16±0.2	17±1.0
Cefazolin	55±1.5	55±1.5	16±0.5	25±0.6	39±0.8	27±0.9	—	25±0.4	—	18±0.2	17±0.4	25±0.3
Ampicillin	40±1.0	40±1.0	16±0.8	—	39±1.0	19±0.3	—	—	18±0.3	—	—	—
Streptomycin	25±0.2	25±0.2	28±1.1	—	8±0.6	24±0.1	12±0.3	—	—	18±0.2	—	—
Kanamycin	25±0.3	25±0.3	25±0.9	—	—	23±0.2	14±0.5	—	—	17±0.2	—	—
Gentamicin	22±0.2	22±0.2	36±1.0	20±1.0	12±0.3	25±0.5	18±0.2	20±0.5	14±0.5	21±0,4	—	—
Erythromycin	45±1.0	45±1.0	15±0.2	—	15±0.4	—	—	—	21±0.5	—	—	—
Tetracycline	40±0.5	40±0.5	18±0.3	—	—	21±0.1	15±0.6	—	—	19±0.3	—	—
Ciprofloxacin	22±0.1	22±0.1	32±1.0	—	39±0.3	23±0.3	27±1.0	18±0.2	—	33±1.0	14±0.3	17±1.0
Amoxicillin with clavulanic acid	50±1,0	50±1.0	—	20±0.4	48±1.2	23±0.3	—	—	24±0.3	16±0.4	22±0.2	—
Oxytetracycline	35±0.5	35±0.5	15±0.2	—	10±1.0	25±0.1	19±0.3	—	—	20±0.5	—	—
Enrofloxacin	35±1.0	35±1.0	30±1.0	20±0.5	39±1.0	26±0.5	24±0.5	18±0.5	19±0.5	24±1.0	22±0.3	22±0.8

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ВИВЧЕННЯ ЧУТЛИВОСТІ ІЗОЛЯТІВ, ВИДІЛЕНИХ ВІД ХВОРИХ ТВАРИН, ДО АНТИБІОТИЧНИХ ПРЕПАРАТІВ ПОРІВНЯНО З ТЕСТ-МІКРООРГАНІЗМАМИ

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Наведені результати дослідження з визначення чутливості музейних тест-культур мікроорганізмів та ізолятів, виділених від хворих тварин, до шести груп антибіотичних препаратів диск-дифузійним методом. Встановлено, що ефективність їх дії на ізоляти значно менша, порівняно з музейними штамами, що пов’язано з формуванням резистентних форм мікроорганізмів.

Ключові слова: *антибіотичні препарати, штами, ізоляти, резистентність*

Изучение чувствительности изолятов, выделенных от больных животных, к антибиотическим препаратам в сравнении с тест-микроорганизмами

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Приведены результаты исследования по определению чувствительности музейных тест-культур микроорганизмов и изолятов, выделенных от больных животных, к шести группам антибиотических препаратов диско-диффузионным методом. Установлено, что эффективность этих препаратов в отношении изолятов значительно меньше по сравнению с музейными штаммами, что связано с формированием резистентных форм микроорганизмов.

Ключевые слова: *антибиотические препараты, штаммы, изоляты, резистентность*