BIOTECHNOLOGY

УДК 636.087.8 : 579

N. M. Al-Hammash, PhD student (BSTU);
A. V. Ignatenko, PhD (Biology), assistant professor (BSTU)

ANALYSIS OF ANTIBIOTICS RESISTANCE OF *E. coli* MICROORGANISMS

The article deals with the problem of microorganisms' resistance to antibiotics at dairy farms and complexes (DFC). The aim of the work – analysis of resistance of *E. coli* to antibiotics used at DFC. As a result we determined the range of resistance of coli-forms to antibiotics used at the dairy complex "Zabolot" of Grodno district. It was shown that received strains of microorganisms *E. coli* have high resistance to the following antibiotics: oletetrin, erythromycin, polymyxin, benzylpenicillin. *E. coli* bacteria remained high sensitivity to ceftriaxon, sisomicin, furadonin, kanamycin, gentamicin. This fact allows to recommend these antibiotics for animals coli-bacteriosis treatment at DFC.

Introduction. The growth of antibiotics resistance of microorganisms is one of the main and unsolved problems in the struggle with pathogenic microorganisms.

The reason of this phenomenon is connected with the prolonged use of the same drugs in medicine and veterinary for treatment of both human beings and animals, and is also associated with a rapid adaptation of microorganisms to antimicrobial substances.

The growth of antibiotic resistance of microorganisms at first was discovered in medicine and later in veterinary. This phenomenon after that was found for other antimicrobial substances [1–5].

Researchers tried to solve this problem on the way of search and synthesis of new antimicrobial substances. Nowadays it is known about 6,000 antibiotics, but it became clear that adaptation of microorganisms to antibiotics is faster than a search of new antimicrobial substances.

The way from discovering, testing to applying a new antibiotic in practice takes 5–10 years even in the best case and costs several million dollars. At the same time in laboratory conditions microorganisms need several weeks for adaptation to new antibiotics. In nature complete adaptation of microorganisms to new antibiotics is observed during 5 years and partial adaptation – during a year or less [3].

According to FAO/WHO report every year 25 thousand people die from the resistance of microorganisms to antibiotics [5].

In veterinary this problem is sharper. Because of antibiotic resistance cows are used not more than 3 years instead of 5 years planned in dairy production. Then millions of animals are not treated but killed for meat [1].

Thus mankind loses the "armaments race" in fighting with microorganisms.

Until the new more effective ways for struggle with antibiotic resistant microorganisms have not been found, one of the main ways of solving this problem is the proper use of antibiotics, timely detection of increasing of microorganisms resistance and alternation of substances used.

The analysis of microbiota resistance at DFC is one of the immediate tasks that provides sanitary and hygienic conditions for high quality milk production.

At present antibiotics resistance control of microorganisms at DFC isn't carried out because of the absence of available methods of analysis. Such work is fulfilled by veterinary and microbiological test centers and research organizations.

Main part. The aim of investigation is to study the resistance of *E. coli* microorganisms to antibiotics used at DFC.

Coli-bacteriosis is one of the widespread reasons of animals disease with mastitis [1]. *E. coli* bacteria belong to opportunistic pathogens and serve as the main sanitary indicative microorganisms, used for characterising safety of foodstuffs. They also are remarkable for high specific growth rate and rapid adaptation to environment [6].

Opportunistic pathogenic microbiota is the obligatory inhabitant of animal organism and is accumulated in great quantity in premises of DFC and constantly accompanies pathogens causing illnesses.

The work with pathogenic microorganisms is difficult because of their low concentration and special conditions while working with them. Therefore it is advisable to carry out monitoring of microorganism resistivity to antibiotics on opportunistic pathogenic microbiota that is easier to find and control.

Research sampling was carried out at the dairy complex "Zabolot" of Grodno district. Isolation, identificaton of microorganisms and their antibiotic resistance analysis was done in the laboratory of Microbiology and Epizootology Department of Grodno State University and at the Department of Biotechnology and Bioecology of BSTU.

Isolation of enterobacteria was done from postmortem animals as described in [7]. Differentiation and identification of microorganisms *E. coli was* fulfilled in accordance with existing systematics accepted by intestinal bacteria subcommittee of nomenclatural committee of International Association of Microbiologists, Bergy's catalogue and immunological method as well [6, 8].

Estimation of *E. coli* resistance to antibiotics was tested by the standard method of substances diffusion into agar [9].

For detection of bacteria sensitivity to antibiotics by disco-diffusion method 0,1 ml of bacteria suspension equal to 0,5 standard turbidity on McFarland was sowed on the agar surface in Petri dishes and then paper discs of 6 mm diameter containing definite quantity of antibiotics, recommended by the instruction on drug application, were put.

After the incubation of plates for 24 hours in thermostat at 37°C diameters of inhibition zones of bacteria growth around discs were measured.

Antibiotics widespread in veterinary for animals treatment were used such as; furadonin, carbenicillin, oletetrin, neomycin, cephtriaxon, benzyl penicillin, oleandomicin, kanamycin, clindomycin, perflocsacin, cephalexin, sizomycin, levomecitin, polymixin, erythromycin, linkomycin, gentamycin, oxacillin, tetracycline, fuzidin, ampicillin.

Working concentrations of antibiotics were prepared from sold medicines in accordance with instruction on their application.

In the result of the fulfilled work 18 cultures of *E. coli* bacteria were isolated and their sensitivity towards antibiotics stated by disco-diffusion method.

The results of sensitivity and resistivity testing of a certain strain of *E. coli* bacteria to separate antibiotics are shown at Fig. 1.

Antibiotic resistance of microorganisms was estimated on a diameter of the inhibition zones of bacteria growth in agar media and was stated in accordance with the commonly accepted criteria (Table 1).

As it is seen from Table 1 if the size of growth inhibition zone is more than 12 mm microorganisms are considered to be sensitive; if less than 12 mm – middle resistant; at the absence of growth inhibition zone – they belong to resistant forms [9].

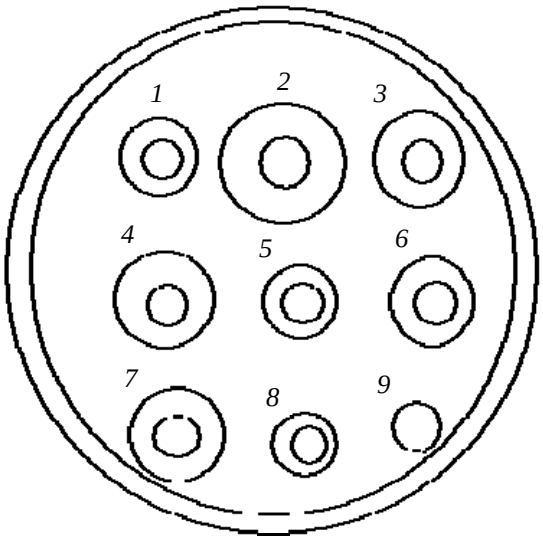

Fig. 1. Analysis of bacteria *E. coli* resistance to antibiotics by the method of substances diffusion into agar media:
1 – benzyl penicillin; *2* – sizomycin; *3* – furadonin, *4* – neomycin; *5* – polymixin; *6* – tetracycline, *7* – kanamycin; *8* – erythromycin; *9* – oletetrin

The results given at Fig. 1 show that selected strain of *E. coli* has high sensitivity to sizomycin, furadonin, neomycin, kanamycin; medium sensitivity to tetracycline, and high resistance to oletetrin, erythromycin, polymixin, benzyl penicillin.

Table 1

Criteria of microorganisms' resistance

Types of microorganisms	Size of inhibition zone of growth, mm	Doses of antibiotics for therapy
Sensitive	more than 12	recommended
Middle resistant	less than 12	maximum admitted
Resistant	absent	ineffective

In Table 2 the results of resistance analysis of 18 selected strains of *E. coli* bacteria towards investigated antibiotics are given. All isolated microorganisms were divided into groups of sensitive, losing sensitivity and stable forms in accordance with criteria of bacteria resistance to antibiotics and their content in percents was determined.

As it is seen from Table 2 all isolated strains were resistant to antibiotics: oletetrin, oleandomicin, oxacillin, clindomycin,. High percentage of stable forms was detected towards benzyl penicillin, erythromycin, linkomycin, fuzidin, tetracycline. For cephtriaxon, furadonin, sizomycin no resistant forms were found.

Table 2

Analysis of antibiotics influence on resistance of *E. coli* microorganisms

Antibiotics	Forms content, %		
	Sensitive	Medium stable	Stable
1. Furadonin	72.2	27.8	0
2. Carbenicillin	16.7	16.6	66.7
3. Oletetrin	0	0	100.0
4. Neomycin	38.9	55.5	56.0
5. Cephtriaxon	100.0	0	0
6. Benzyl penicillin	0	5.6	94.4
7. Oleandomicin	0	0	100.0
8. Kanamycin	78.6	14.3	7.1
9. Clindomycin	0	0	100.0
10. Perflocsacin	62.5	0	37.5
11. Cephalexin	71.4	0	28.6
12. Sizomycin	88.9	11.1	0
13. Levomecitin	38.9	16.7	44.4
14. Polymixin	16.7	50.0	33.3
15. Erythromycin	0	0	94.5
16. Linkomycin	5.5	0	94.5
17. Gentamycin	83.3	11.1	5.6
18. Oxacillin	0	0	100.0
19. Tetracycline	11.1	5.6	83.3
20.Fuzidin	11.1	0	88.9
21. Ampicillin	33.3	5.6	61.1

Conclusion. It has been fulfilled the analysis of antibiotics resistance of *E. coli* bacteria to the range of widely used antibiotics. Received data make it possible to recommend changing of ineffective antibiotics for effective ones, such as cephtriaxon, furadonin, kanamycin, sizomycin, gentamycin for prophylaxis and treatment of animal coli-bacteriosis at dairy farms and complexes.

References

1. Богуш, А. А. Мастит коров и меры его профилактики / А. А. Богуш, В. Е. Иванов, Л. М. Бородич; рец. П. А. Красочко, М. В. Якубовский, Н. Ю. Щурова. – Минск: Белпринт, 2009. – 158 с.

2. Сидоренко, С. В. Происхождение, эволюция и клиническое значение антибиотикорезистентности // Антибиотики и химиотерапия. – 1999. – Т. 44, № 12. – С. 19–22.

3. Amyes, S. B. Antibiotic resistance / S. B. Amyes, C. G. Gemmel // J. Med. Microbiol. – 1997. – Vol. 46, No. 6. – P. 436–470.

4. Hancock, R. E. The role of fundamental research and biotechnology in funding solutions to the global problem of antibiotic resistance / R. E. Hancock // Clin. Infect. Dis. – 1997. – Vol. 24, No. 1. – P. 148–150.

5. Борьба с устойчивостью к антибиотикам с позиций безопасности пищевых продуктов в Европе / под ред. H. Kruse, F. Racioppi. – Рим: ВОЗ, 2011. – 80 с.

6. Инфекционные болезни и эпидемиология: учеб. для вузов / В. И. Покровский [и др.]. – М.: ГЭОТАР-МЕД, 2004. – 816 с.

7. Солонеко, А. А. Практикум по микробиологии / А. А. Солонеко, А. А. Гласкович, Ф. Е. Тимофеев. – Минск: Дизайн ПРО, 1998. – 192 с.

8. Иммунология: учеб. пособие / П. А. Красочко [и др.]; под ред. П. А. Красочко, Н. Д. Лисова. – Минск: Аверсэв, 2005. – 128 с.

9. Молоко и молочные продукты. Микробиологические методы определения наличия антибиотиков: ГОСТ Р 51600–2010. – Введ. 01.01.2011. – М.: Стандартинформ, 2010. – 11 с.

Received 17.03.2012