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Composition and antibiotic susceptibility of opportunistic pathogenic microflora in poultry farms aimed at egg or meat farming

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***Abstract*— There are a lot of factors facilitating antibiotic resistance in poultry farming, such as: genetic characteristics of microbiome, immune status of poultry, stress-producing technology of industrial poultry welfare, and the use of antibiotics with the purpose of disease prevention or medication. In both veterinary and animal-farming practice in the Russian Federation ansamycins, carbapenems, semisynthetic penicillins, cephallosporins of different generations, fluroquinolones, macrolides and azalides, tetracyclines and glycopeptides are mainly used. Detection of the strains of pathogenic and opportunistic pathogenic microflora resistant to these antibiotics is considered to be important not only for veterinary but also for medicine. We have done the research on opportunistc microbiocenosis in poultry farms aimed at egg or meat farming located in the Ural area. It was stated that dominating strains in opportunistic pathogenic microflora are Enterococcus faecium and Pseudomonas aeruginosa, whereas S. aureus, S. epidermidis, S. saprophyticus, Ent faecalis, Enterobacter spp, P. vulgaris, P. mirabilis, E.coli are less frequently detected. Proportion of the above mentioned microorganisms differed depending on the profile of a poultry farm. The research done have shown that spreading of antibiotic resistant strains of opportunistic microflora in poultry farms have reached high level. The most of detected strains had low susceptibility or resistance at least to one target antibiotic. A significant number of P.aeruginosa and S.aureus strains not sensitive to carbapenems and fluroquinolones, as well as of E. faecium strains resistant to vancomycin, have been detected.**

Keywords*— *meat poultry farming, egg poultry farming, opportunistic pathogenic microflora, antibiotic susceptibility, strains.

I. Introduction

Modern industrial highly-productive poultry farming causes high stress level that negatively affects animal health. Close contact of poultry, room limitations and mobility restrictions, as well as improper diet not suitable for metabolic demands of poultry result in reduction of protective functions and spreading of microorganisms [1]. Weakening of immune system leads to the stock infection with purulent-septic pathology of various organs and systems that need antibiotics treatment. Nowadays the use of antibiotics is forced necessity, in order to control stock disease incidence, as now antibiotics treatment is the simplest and most effective way to struggle with pathogenic and opportunistic bacteria. A number of countries widely use preventive antibiotics, that is antibiotic supplements into diets of animals and poultry that has a stimulating and protective effect. In poultry farming they are used for stimulating egg-producing ability and growth of muscle mass, reduction of incidence of disease, and others. Most developed countries refuse from and legally ban the use of feed antibiotics. However, dishonest manufacturers have been still using them, in order to balance technological errors. Thus, all the above-mentioned leads to creation of favourable conditions to form low-sensitive and resistant strains of microorganisms in poultry farms. The typical representatives of opportunistic microflora that are most quickly adaptive to anti-infective drugs are S. aureus, P, aeruginosa, and Enterococcus. Individual microbiocenosis of poultry organism also significantly affects their health [2]. In case of contamination of young birds with resistant strains of microflora, risk of treatment-resistant bacterial diseases and dysbacteriosis increases. One of dangers caused by spreading microbial resistance in animal and poultry farms is possibility of contamination of staff and exposed people with resistant strains of opportunistic pathogenic microflora [3]. In both veterinary and animal-farming practice in the Russian Federation ansamycins, carbapenems, semisynthetic penicillins, cephallosporins of different generations, fluroquinolones, macrolides and azalides, tetracyclines and glycopeptides are most often used. Loss of susceptibility to them by microorganisms presents risk for human health, as these groups of medications are also used in medicine for treatment of infectious, surgical and purulent-septic pathology. Consequently, detection of strains of pathogenic and opportunistic pathogenic microflora resistant to the antibiotics (especially Pseudomonas aeruginosa, Staphylococcus spp., Enterococcus and others) and research on mechanisms of their formation in animal farming is important not only for animals, but also for human health.

II. Aim

The aim of the work was analysis of typical pathogenic and opportunistic pathogenic microflora in various objects of industrial poultry farming in a few regions of the Russian Federation. In order to achieve the aim, the following tasks were set: to do analysis of genus-species composition of opportunistic microflora in poultry farms aimed at egg or

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meat farming; to evaluate susceptibility of detected strains to antibiotics mostly used in the industry.

III. Materials and Methods

We have done the research on genus-species composition of microbiota and its antibiotic susceptibility in poultry farms aimed at egg or meat farming located in the Ural area. The enterprises were selected according to their location, structure, commercial-economic value, and economic relations with other enterprises in neighbouring regions and areas. The farms under research had similar technological and production cycles, birds' crosses, performance data, and homotypic diet and veterinary maintenance. Totally, eight enterprises have been researched.

Genus-species composition of microbiota in different places have been researched. The samples of feeding stuff, water, air, litter, poultry manure, and wash-off from cages, feed-throughs, drinking pans and tools for birds' maintenance were taken; wash-off from eggs and dung tape were made; wash-off from mucosa, feather and skin cover, and from cloaca of laying chicken (in the poultry farms aimed at egg farming) and broiler chicken of antemortem age (in the poultry farms aimed at meat farming) were made. In total, 456 samples were taken.

The materials were analyzed according to standard microbiological methods: inoculations of medium, cultivation, recovery of pure line, identification of microorganisms, and evaluation of their antibiotic susceptibility by disk-diffusion method (Minimum Inhibitory Concentration) and serial dilution method [4]. The research was done on s susceptibility of microorganisms to the types of antibiotics mostly often used in veterinary practice and feeding in the region: rifampicin (ansamycins), meropenem (carbapenems,betalactams), ampicillin, amoxicillin (semisynthetic penicillins), cefazolin (cephallosporins of the 1st generation), enrofloxacin (fluroquinolones), azithromycin (macrolides and azalides), tetracycline (tetracyclines) and vancomycin (glycopeptides). For screening assessment of birds' immune system in all the enterprises under research blood samples for immunological test and hematology profile were taken (total blood score, quantity of TC- and B-lymphocytes, immunoregulatory index, phagocytosis indexes, circulating immune complexes, osmoresistance of erythrocytes and activity of blood lipid peroxidation were defined).

The plan, methods, technology of the research and data processing algorithm were approved by the ethics committee and Measurements and Standards Committee.

IV. Results of the Research and Consideration of Them

The research have shown that the dominating microorganism detected in 63-100% of samples was Enterococcus faecium. The second one according to frequency of detection was Pseudomonas aeruginosa detected in 29-100% of samples depending on an enterprise under research. Staphylococcus aureus was also detected in samples taken from all the enterprises under research, frequency of detection was 8-65% on average, whereas in some enterprises Staphylococcus aureus was detected in every sample taken (100%). Frequency of detection of strains of other microorganisms varied depending on an enterprise under research. Also, some weak tendencies in changing structure of microbiota depending on technology of birds' breeding were noticed.

In poultry farms aimed at meat farming typical structure of opportunistic pathogenic microflora was the following: Ent. faecium (32,2%), S. aureus (30,6%), P. aeruginosa (18,6%), S. epidermidis (5,2%), C. albicans (4,7%), Proteus spp. (2,8%), Aspergillus spp. (2,6%), E.coli (2,2%). Other microorganisms (S. saprophyticus, Enterobacter and others) made up less than 1% in total. Poultry farms aimed at egg farming had somewhat different structure of opportunistic microbiota. Typical distribution of the detected strains according on frequency of detection was the following: Ent. faecium (23,9%), P. aeruginosa (19,7%), E.coli (13,9%), S. aureus (11,4%), Aspergillus spp. (9,3%), S. epidermidis (9,2%), Enterobacter spp. (8,9%), C. albicans (2,7%), Proteus spp. (0,5%), and other microorganisms made up less than 0,5% in total. It is worth to note high frequency of detection of E.coli in wash-offs, as well as the fact that frequency of detection of S. aureus strains was twice less. At the same time, proportion of the samples with detected Pseudomonas aeruginosa was comparatively similar in all the enterprises under research (18-20%).

In wash-off from oral cavity mucosa of laying and broiler chicken S. aureus, P. aeruginosa, Ent. faecium, S. epidermidis,and E.coli were mostly detected. In wash-off from birds' cloaca – mostly Ent. faecium, E.coli, Enterobacter spp. (nonpathogenic strains), Aspergillus spp., and P. aeruginosa. In wash-off from cages, drinking pans and tools Ent. faecium, S. aureus, S. epidermidis, P. aeruginosa, as well as C. albicans were most often detected.

Evaluation of antimicrobial resistance of the detected microorganisms.

While evaluating antibiotic susceptibility of strains, we considered only the microorganisms affected by antibiotic spectrum, that are its targets influenced by antibacterial and bacteriostatic effect of the medication in Minimum Inhibitory Concentration. Microorganisms with natural resistance to some definite type of antibiotics were not taken into consideration. Cases of dose-dependent action, native moderate and weak antibiotic susceptibility of microorganisms were not researched.

The research on antibiotic susceptibility of strains from samples taken in poultry farms have shown that more than 40% of detected strains are resistant at least to one from nine used medications. At the same time, multiresistant (resistant at least to three types of antibiotics) strains made up almost 6%. Most quantity of resistant strains were detected in S. aureus - 65% of the total number. The least quantity – in Proteus spp. – 1,4%. Generally, most cases of resistance was: to macrolides – 24% of the detected strains were resistant; to fluroquinolones - 16,7% and to tetracyclines – 9,3%. Highest susceptibility of microbiota was stated to rifampicine (1,1% of resistant strains in total) and to glycopeptides (1%).

It is worth to note that there is a significant number of S. aureus strains that lost susceptibility to beta-lactam antibiotics. Thus, in samples from poultry farms aimed at egg farming, proportion of S. Aureus resistant to ampicillin and amoxicillin was 33%, and to meropenem - 18% (Fig. 1).

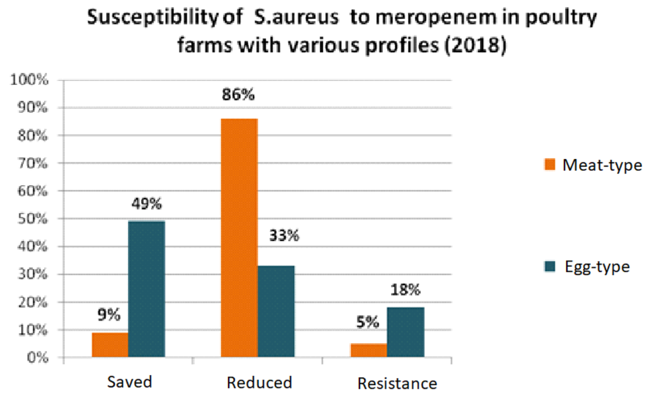

Fig. 1. Proportion of S. Aureus strains with various levels of susceptibility to meropenem in poultry farms aimed at meat or egg farming

In P. aeruginosa resistance to meropenem was detected only in strains detected in poultry farms aimed at meat farming (9% of all the detected P. aeruginosa strains on average). In poultry farms aimed at egg farming no P. aeruginosa strains resistant to meropenem were detected. Analysis of susceptibility of this microorganism to fluroquinolones (enrofloxacin) has shown that generally all the objects had strains with reduced susceptibility to enrofloxacin.). In poultry farms aimed at meat farming no P. aeruginosa strains with good susceptibility to enrofloxacin were detected. In poultry farms aimed at egg farming their proportion was 20% of all the detected microorganisms of that type on average (Fig. 2).

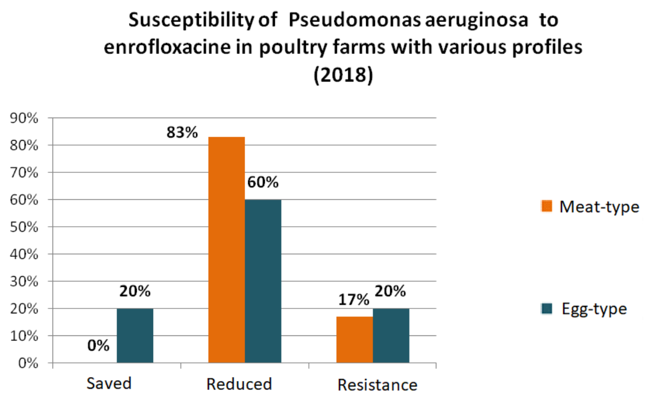

Fig. 2. Proportion of P. aeruginosa strains with various levels of susceptibility to enrofloxacine in poultry farms aimed at meat or egg farming

E. faecium strains detected in samples were researched on susceptibility to vancomycin, in order to identify VRE-strains that are most dangerous for animals' and human health [5]. It was stated that averagely 50% of detected Enterococcus strains in poultry farms aimed at egg farming and 85% in poultry farms aimed at meat farming had reduced susceptibility to this antibiotic. At the same time proportion of resistant strains in samples from poultry farms aimed at egg farming was higher. No E. faecium strains with good susceptibility to vancomycin in researched poultry farms aimed at meat farming were detected (Fig. 3).

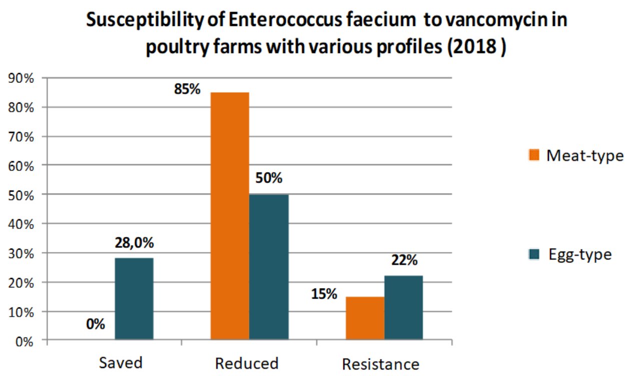

Fig. 3. Proportion of Ent. faecium strains with various levels of susceptibility to vancomycin in poultry farms aimed at meat or egg farming

Analysis and generalization of the data obtained allow to make a conclusion about acquired feature of antibiotic resistance of Ent. faecium, P. aeruginosa, and S. aureus strains. Multiresistant strains of Staphylococcus and P. Aeruginosa were subjected to additional analysis, in order to determine probable mechanisms of stability (tests with oxacilline and Betalactamase inhibitors). It was stated that more than 60% of all the multiresistant P. aeruginosa strains detected in the course of research, had low susceptibility or resistance to carbapenems, fluroquinolones, macrolides and tetracyclines. Presumably, it is caused by the fact that these strains have plasmid betalactamases of D-type (Gram-negative bacteria) able to hydrolyze penicillins, and specific transport systems removing carbapenems from a bacterial cell, as well as modified topoisomerase, which are non-sensitive to fluroquinolones [6-9]. In general, obtained results prove that the identified strains have acquired resistance of plasmid or chromosomal nature effected by various mechanisms and their combinations.

Research on hematologic and immunological indicators of natural resistance of chicken bred in poultry farms under research, have identified the signs of reduced systemic immune response. Decreased defence and adaptative response of birds' organism is primarily explained by selection aimed at higher productivity, as well as by industrial technology of birds' welfare [2]. All the birds under research had disfunction of phagocytosis. Quantity of engulfing leukocytes averagely increased up to 42,3% (normally 12-25%), whereas performance of phagocytes decreased up to 2,12 m.b. (normally 2,5-6,0 m.b.). Decrease of CIC level in blood serum up to 75,6 c.u. was stated. These data, taking into account physiological and species characteristics of birds, prove suppression of non-specific component of immune system.

V. Conclusion

The research have shown that dominating representative of opportunistic microflora in poultry farms were Enterococcus faecium and Pseudomonas aeruginosa detected in most samples. Also, S. epidermidis, S. saprophyticus, Ent faecalis, Enterobacter spp, P. vulgaris, P. mirabilis, E.coli, as well as C. albicans and Aspergillus spp. were often detected.

Proportion of these microorganisms in the structure of opportunistic pathogenic microflora in researched poultry farms aimed at egg or meat farming had some differences: in particular, frequency of detection of S. aureus differed more than twice, and of E.coli – more than five times. Proportion of samples where Pseudomonas aeruginosa was detected was comparatively similar in all the poultry farms under research. Susceptibility of most microorganisms detected in samples to target antibiotics can be characterized as reduced. More than 40% of identified strains were resistant to one of nine antibiotics under research. In samples taken at poultry farms aimed at egg farming proportion of S. aureus resistant to ampicillin and amoxicillin, was 33%, and to meropenem - 18%. P. Aeruginosa strains resistant to meropenem were detected in poultry farms aimed at meat farming, and resistant to enrofloxacin – in all the poultry farms under research. E. Faecium strains in all the poultry farms under research had low susceptibility to vancomycin minimum in 50 % of cases. Vancomycin-resistant Enterococcus were more often detected in detected in poultry farms aimed at egg farming. Research on hematologic and immunological indicators of chicken have shown signs of reduced systemic immune response and suppression of non-specific component of immune system.

The research have shown that spreading of antibiotic resistance strains of opportunistic microflora in poultry farms have reached high level. Generally, it can be said that a number of antibiotics used in poultry farming have lost their effectiveness against target microorganisms. Increased resistance is facilitated by not only internal factors of microbiome, but also external factors, such as: reduced immune status of birds, stress-producing technology of industrial poultry welfare, and the use of antibiotics with the purpose of disease prevention or medication.

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