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Profile of antibiotic susceptibility of enterococcus in pig farms

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***Abstract*—The research on the structure of opportunistic pathogenic microflora of pig farms with various technological characteristics has been done. The research was done on content of Enterococcus spp. in composition of opportunistic pathogenic microbiocenosis, dissemination of various objects, as well as profiles of antibiotic susceptibility of detected strains of enterococcus. The analysis of interaction between quality and balance of pigs' diets and prevalence rate of resistance strains of Enterococcus faecium Enterococcus faecalis in enterprises has been done. In the course of the research we have identified the structure of microflora with prevailing content of Ent. faecium, P. aeruginosa, S. aureus, which is typical for pig farms. Dissemination if various objects with enterococcus was different, whereas the highest one was identified among pregnant sows. Critically high level of antibiotic susceptibility of enterococcus in all the enterprises has been stated. It was stated that almost half of all the detected enterococcus were resistant to one antibiotic. Multiresistant as well as vancomycin-resistant (VRE) strains of Ent. faecium and Ent. faecalis were detected in the enterprises where diets of lower quality were used.**

***Keywords*— pig-farming, opportunistic pathogenic microflora, Enterococcus, antibiotic susceptibility, resistance.**

I. Introduction

Nowadays reduction of antibiotic susceptibility of microorganisms is considered to be one of burning issues in medicine, veterinary and animal farming [1,2]. Spread of microbial resistance occurs faster and faster, and antibiotics are getting non-effective quickly, and in veterinary and clinical practice incurable cases of bacterial infection resistant to any scheme of antibiotic treatment take place more often. Health care centres have the system of monitoring resistant strains and the use of antibiotics, as well as the treatment schemes, in order to reduce the risks of development of microbial resistance [3,4]. In animal farming and veterinary of the most countries strategies of microbial resistance control are not well developed. Such factors, as absence of monitoring of susceptibility of microorganisms, the use of feed antibiotics for prevention and stimulation, irrational selection of treatment medications, high level of stress and reduced immune resistance of organism under the conditions of industrial animal breeding, overpopulation and favourable conditions for spreading of strains, result in quick adaptation of opportunistic microflora to external influence, including the use of antibiotics [4,5]. In Russia productive animals annually consume about 3,500,00 tons of antibiotics, whereas 19% of them as growth-promoting substances, and 22% - for prevention measures [6]. Antibiotics are often used to correct technological errors, in such cases as: imbalanced diet, low quality feedstuff, high level of disease incidence, decrease of productivity and others. Under the conditions of constant contact with low doses of antibiotics microorganisms lose their sensitivity especially quickly. The most adaptive ones are such typical representative of opportunistic microflora as S. aureus, P, aeruginosa, E. coli and various types of enterococcus. Despite of the fact that enterococcus are included in normal microflora of humans and some mammals, multiresistant and vancomycin-resistant strains (VRE) are considered to be the most dangerous ones, as they can cause severe enteritis, bacteriemia and sepsis among humans and animals having immunodeficiency of physiologic (neonatal age) and pathologic nature (infectious, oncological, toxical and others) [7,8,9,10]. Personnel of pig farms and other people that are in constant contact with animals are subject to high level of risk of being contaminated with resistant enterococcus strains, that may have a negative effect on their health in case of developed opportunistic infections [2]. In order to solve the above-mentioned problem, it is necessary to modify existing veterinary and technological strategies in pig farming, which are used to control microbial resistance. Some system, including better control over the opoortunistic pathogenic microflora at enterprises, certificates of resistance of veterinary objects, better use of antibiotics, rejection of feed supplements including antimicrobial drugs, as well as development of new methods of treatment and prevention, which reduce demand of animal farming on antibiotics, are needed to be developed. Thus, it is considered to be important to do the research on modern profile of antibiotic susceptibility of enterococcus in pig farms with various specific methods of technology.

II. Aim

The aim of the work was to do the research on content of Ent. faecium in microbiocenosis of pig farms in the Ural area, as well as the analysis of susceptibility of detected strains to antibiotics mostly used in this industry.

III. Materials and Method

Research was done on generic and specific composition of microbiota; antibiotic susceptibility of detected enterococcus strains in pig farms located in the Ural area. The farms were selected according to their technological and production system, parameters of productivity, location, structure and logistical connections. All the pig farms were divided into two groups depending on the level of balance of

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pigs' diet. The first group was characterized by high quality feedstuff and full-value diet for various groups of pigs, which met pigs' physiological needs. The second group included the pig farms with improper animal nutrition, imbalanced diet and low-quality feedstuff. 8 enterprises in total have been researched. In the pig farms samples of air, feedstuff and premixes, litter, water for animals were taken and wash-offs from mucosa and mamilla of pregnant and farrow sows, as well as from mucosa and skin of piggery from weaning cohort, nursery and fattening groups were done; wash-offs from equipment, fences, surfaces and tools in different places of premises. Totally, 495 samples have been taken.

The material was researched according to standard microbiological methods: inoculation of medium, cultivation, detection of pure line, identification of microorganisms and determination of their antibiotic susceptibility by means of diffusion method (Minimum Inhibitory Concentration) and serial dilution method [11]. While determining antibiotic susceptibility of enterococcus strains only the medications that have bactericidal or bacteriostatic effect on Enterococcus spp. in Minimum Inhibitory Concentration were taken into account. Cases of dose-dependent action, and native moderate or low antibiotic susceptibility were not considered. The research was done on enterococcus sensitivity to rifampicin (ansamycins), meropenem (carbapenems), ampicillin, amoxicillin (semisynthetic penicillins), enrofloxacin (fluoroquinolones), azithromycin (macrolides and azalides), tetracycline (tetracyclines) and vancomycin (glycopeptides).

Plan, methods, research technologies and data processing algorithms are approved by the ethic committee and Committee of Metrology and Standartization.

IV. Results and Discussion

Research done in pig farms of both groups have shown that specific structure of opportunistic pathogenic parts of microbiota is relatively similar. Some differences were identified only in proportions of mold fungi. The dominating pathogenic and opportunistic pathogenic bacteria in the samples were Ent. faecium, P. aeruginosa, S. aureus (Fig. 1).

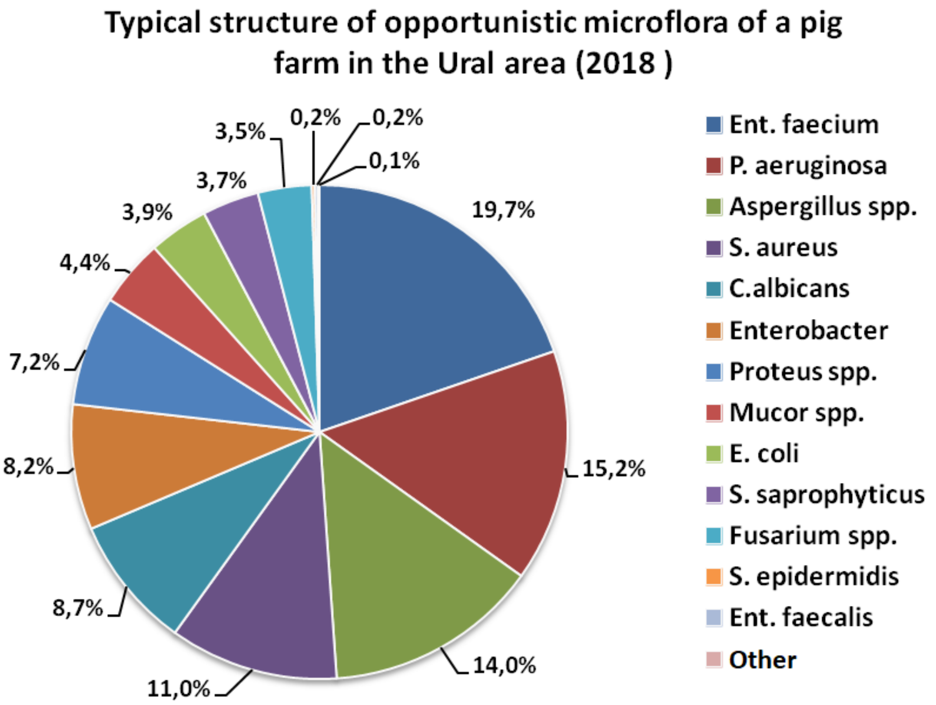

Fig. 1. Typical structure of opportunistic microflora of a pig farm (according to the results of research on eight pig farms in the Ural area, 2018)

High level of pregnant sows' contamination with Ent. faecium, C. albicans, S. aureus and P. aeruginosa was stated: more than a half of wash-offs from tunica mucosa of mouth, nasal mucosa and mucosa of vagina proved to be positive on those microorganisms. Tools in all the enterprises under research was contaminated with mostly Ent. faecium and S. aureus, as well as with P. aeruginosa and fungal microflora. Total bacterial count in air samples from premises of pig farms was from 1800 to 4926 CFU/m3 that verified high microbial challenge.

Analysis of Ent. Faecium susceptibility to various types of antibiotics has shown that averagely 43% of all the detected strains were insensitive at least to one medication (more often to tetracyclines, macrolides and semisynthetic penicillins). Also, multiresistant enterococcus strains resistant to three and more types of antibiotics were detected. In samples taken at the enterprises of the first group they made up 2% of all the cases of resistance; at the enterprises of the second group - 5,4%. Multiresistant enterococcus strains were identified in wash-offs from mucosa of vagina and tunica mucosa of mouth of breeding pigs and piggery from weaning cohort and nursery group, as well as from samples of litter and wash-off from tools in the premises where those animals were kept.

In general, enterococcus detected in samples at the enterprises of both groups have shown mostly low susceptibility to the antibiotics under research.

Differences between two groups of enterprises were seen in total number of strains resistant to various kinds of antibiotics. Thus, in pig farms of the second group, more enterococcus strains, which lost their susceptibility to rifampicin, tetracycline, meropenem, amoxicillin, and vancomycin were detected, as compared with the samples from enterprises of the first group (Tables 1,2).

TABLE I. PROPORTION OF ENTEROCOCCUS SPP. STRAINS WITH RESISTANCE, LOW AND GOOD, ANTIBIOTIC SUSCEPTIBILITY IN PIG FARMS FROM GROUP #1

Antibiotic	Antibiotic susceptibility of Enterococcus spp. (pig farms of Group 1)		
	Preserved	Reduced	Resistance
Rifampicin	31%	69%	0%
Meropenem	25%	75%	0%
Amoxicillin	50%	45%	5%
Tetracycline	6%	81%	3%
Enrofloxacin	37%	63%	0%
Azithromycin	19%	69%	12%
Ampicillin	68%	25%	7%
Vancomycin	25%	75%	0%

Parameters of enterococcus susceptibility to azithromycin were higher in enterprises of the second group – proportion of resistant strains in the total number of all the detected enterococcus was 7%; in enterprises of the first group – averagely 12% (Table 2).

TABLE II. PROPORTION OF ENTEROCOCCUS SPP. STRAINS WITH RESISTANCE, LOW AND GOOD ANTIBIOTIC SUSCEPTIBILITY IN PIG FARMS FROM GROUP #2

Antibiotic	Antibiotic susceptibility of Enterococcus spp. (pig farms of Group 2)		
	Preserved	Reduced	Resistance
Rifampicin	13%	80%	7%
Meropenem	40%	53%	7%
Amoxicillin	46%	33%	21%
Tetracycline	27%	67%	6%
Enrofloxacin	33%	67%	0%
Azithromycin	20%	73%	7%
Ampicillin	66%	26%	8%
Vancomycin	20%	67%	13%

Most strains resistant to macrolides were sensitive to all the rest of antibiotic researched. Such cases of Enterococcus spp. monoresistance to macrolides were probably caused by that strains have enzyme inactivation mechanisms (production of macrolidephosphotransferases and others) or active removing [3,4,7]. In general, the obtained results prove that detected enterococcus strains have acquired resistance of plasmid or chromosome nature realized through various mechanisms or combination of them.

Enterococcus susceptibility to vancomycin is of special significance. Spread of vancomycin-resistant strains presents great risk and needs to be under special control at animal farms [2,3]. Analysis of susceptibility of detected Ent. faecium strains to vancomycin has shown that samples from enterprises from group #1 did not have any strains of vancomycin-resistant enterococcus. At the same time, ¾ of the strains had low susceptibility, and 25% - good susceptibility to vancomycin. In samples from group #2 averagely 13% of strains were resistant to vancomycin (Fig 2).

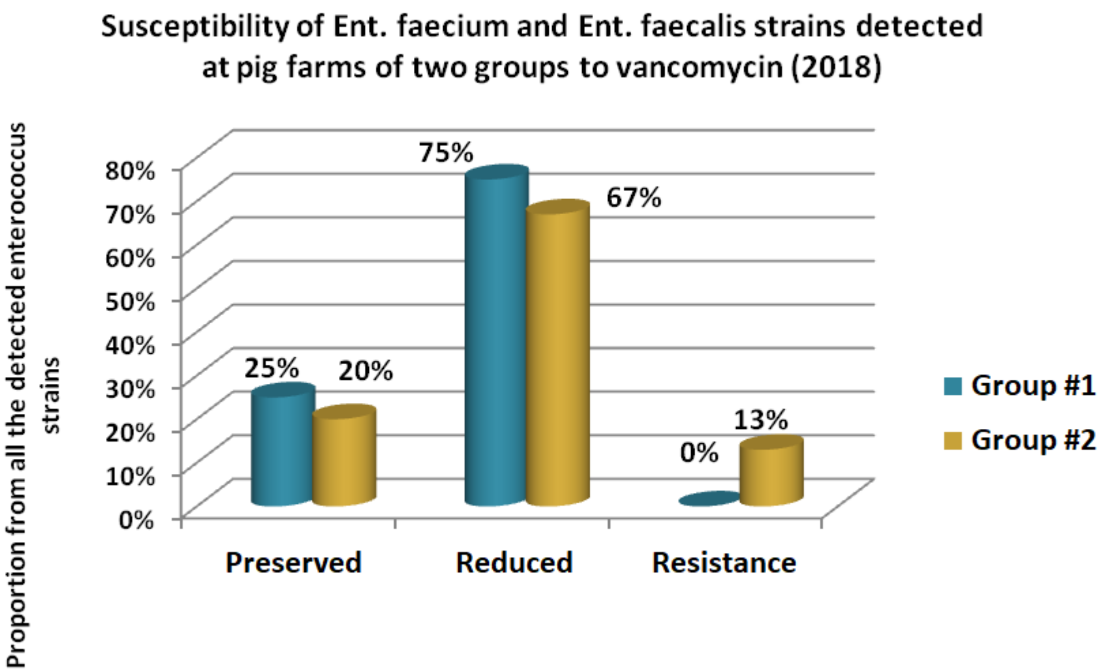

Fig. 2. Susceptibility of Ent. faecium and Ent. Faecalis strains detected at pig farms of two groups to vancomycin (2018)

Combination typical for VRE "carbapenems + vancomycin" in profile of antibiotic susceptibility [1] was detected at enterprises of group #2. 11% of all the detected Ent. faecium strains were resistant both to meropenem and vancomycin. At enterprises of group #1 no such strains were detected. At the same time low susceptibility to these two antibiotics was detected among more than a half of enterococcus in both groups of enterprises.

V. CONCLUSION

The research has shown that structure of an opportunistic pathogenic part of microbiota at pig farms under research was relatively similar. Dominating microorganisms in most of the objects were enterococcus, P. aeruginosa, and S. aureus. The highest level of contamination with enterococcus was stated among pregnant sows – more than a half of wash-offs from mucosa had those microorganisms. Also, most enterococcus were detected in wash-offs from tools and equipment. In general, almost a half of all the detected enterococcus were insensitive to one antibiotic. At the same time, at enterprises using lower quality diet, multiresistant Ent. faecium and Ent. Faecalis strains resistant to three or more types of antibiotics were detected twice more often. At the same enterprises vancomycin-resistant and vancomycin + meropenem-resistant strains were detected. At enterprises using higher quality and balanced diet for pigs, no such strains were detected. Thus, it is possible to make a conclusion that level of antibiotic susceptibility of Enterococcus spp. in pig farms of the region is critically high. One of the factors facilitating spread of resistant strains is low quality and imbalanced diet of pigs.

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