Biofilms of pathogenic bacteria in pig industry

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In the industrial pig farm for 26 thousand heads, the analysis of the influence of a forage factor on bacteria carriers of a uterine pig population in connection with mass morbidity of dairy piglets on anaerobic enterotoxemia is carried out. *Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria spp., Candida albicans, Aspergillus niger* which are able to form biofilms, were isolated from five samples of “SK-1” compound feed for pregnant sows and from the blood of animals (n=20) fed with this compound feed. The structural basis of the most stable biofilms *in vitro* were the aerobic fungi *Aspergillus niger* and *Candida albicans*. Biofilm-forming variants of these bacteria showed multidrug resistance to 30 antimicrobial drugs (synthetic penicillins, cephalosporins, fluoroquinolones, aminoglycosides, tetracyclines, combination drugs). Isolates of associative microflora isolated from the blood of sows were pathogenic for 30% of laboratory mice. It was found that probiotic agent No1 (composition based on Bischofite with probiotics) showed the universal bactericidal activity against the bacteria *Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria spp.*

**Keywords**: biofilms, compound feed, microbial contamination of feed, multiresistance, sows.

**Introduction**

The development of the pig industry on the basis of concentration, specialization and agro-industrial integration with the introduction of industrial technologies is a natural process for all developed countries. The profitability of the industry directly depends on the epizootic well-being and productive characteristics of the livestock. Low productivity, morbidity and death of farm animals are often associated with contamination of raw materials and feed by pathogenic microorganisms (salmonella, pasteurellosis, listeria, hemophilia, enteropathogenic types of *Escherichia coli*, clostridia), which pose a threat to the health of not only animals but also humans (Crump et al., 2002; Bintsis, 2018). Despite the successes of recent years in optimizing the feeding of farm animals, the role of microbial contamination of feed in reducing production efficiency requires additional attention and careful and comprehensive study (Maciorowski et al., 2007; Mahami et al., 2019). Quantitative and qualitative composition of the microflora of pig feed is very diverse and is formed under the influence of many factors (Pereyra et al., 2010). First of all, if the feed is a product of processing vegetable raw materials, epiphytic microflora of raw materials is largely transferred to the finished product, as well as microorganisms on the processing equipment, in storage shops, warehouses and so on. A special group consists of phytopathogenic microorganisms, including mold fungi (Donlan & Costerton, 2002; Flemming & Wingender, 2010). They affect plants in the field and can produce diverse in chemical nature mycotoxins (Shirokikh et al., 2017).

In recent years, laboratory studies of microbial contamination of feed have shown that a significant amount of animal feed did not meet the requirements of regulatory documentation by the degree of contamination by pathogenic microflora, microscopic fungi, including live saprophytic, pathogenic and opportunistic microorganisms (Pereira et al., 2019). Bacterial associations isolated from feed are characterized by a high level of antibiotic resistance and have the ability to withstand adverse environmental factors (Abdullahi et al., 2016), such as temperature changes, acidity changes, the presence of bactericidal substances and even disinfectants. Their presence in feed is directly related to chronic diseases of animals of productive species, so sanitary requirements for animal feed should be as strict as for food (Tamang et al., 2016; Ali et al., 2018; Machado-Moreira...
Materials and methods

Microbiological studies of feed were performed in the laboratory for swine diseases of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv) using modern methods (Quality Control for Feed Safety at the International Level International Feed Safety Alliance (IFSA), 2018). The ability of microorganism isolates to form biofilms was studied by current methods (O'Toole & Kolter, 1998). Determination of the sensitivity of microorganisms to antimicrobial drugs was performed by the disco-diffusion method (Guidelines for Susceptibility Testing of Microorganisms to Antibacterial agents, 2018).

Sampling and delivery of compound feeds for pigs ("SK-3", "SK-5", "SK-6", "SK-31" for rearing and "SK-1" for pregnant sows) in the amount of 36 samples was carried out from a standard pig farms of Dnipropetrovsk region for 26 thousand heads, which is unfavorable for infectious pneumoenteritis (suckling pigs suffer from anaerobic enterotoxemia, animals for rearing and fattening have an associated course of pasteurellosis and hemophilosis).

Compound feeds for animals are made from the farm's own raw materials and are stored for no more than 7 days. Blood was collected from 20 animals fed with contaminated feed for microbiological testing for bacterial agents (SOP: Blood Collection for Clinical Use, VA, 2019). Materials and methods of Experimental and Clinical Veterinary Medicine (Kharkiv) using modern methods (Quality Control for Feed Safety at the International Level International Feed Safety Alliance (IFSA), 2018). The ability of microorganism isolates to form biofilms was studied by current methods (O'Toole & Kolter, 1998). Determination of the sensitivity of microorganisms to antimicrobial drugs was performed by the disco-diffusion method (Guidelines for Susceptibility Testing of Microorganisms to Antibacterial agents, 2018).

Isolation, cultivation and study of cultural, morphological properties of feed microorganisms were carried out on nutrient media: peptone broth (PMB) with a pH of 7.2-7.4; Hottinger broth; Martin's medium; 2.5% PMB with the addition of 2.0% glucose or selective Fraser supplement (to isolate listeria); meat-peptone agar (MPA) with a pH of 7.2-7.4; Endo agar; modified Kitt-Tarozzi medium; Blaurok's medium; agar Saburo; Olkenytsky's medium; Simons citrate; acetate agar; PALKAM agar (for identification of listeria); Mueller-Hinton agar for disco-diffusion test (DDT).

The pathogenicity of isolated field isolates of bacteria was tested on white mice weighing 16-18 g by intra-abdominal injection at a dose of 0.5 billion microbial bodies, in compliance with the Law of Ukraine "On protection of animals from cruel treatment" (No1759-VI of 15.12.2009) and norms of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes" (Strasbourg, France, 1985). Ethyl ether was used for anesthesia.

For the destruction of biofilms in forages we used drugs produced by LLC 'Sirion' (Dnipro):
- Prebiotic agent – a composition based on Bischofite (chloride-magnesium complex);
- Probiotic agent No1 – composition based on Bischofite with probiotics;
- Probiotic No 2 – concentrate of probiotic bacteria and fungi for feed disinfection.

The results were taken into account for the presence of bacterial growth and their number in colony-forming units (CFU) compared to the control. The number of mesophilic aerobic and facultative-anaerobic microorganisms (total microbial number) (MAFAm) was also determined.

Results and discussion

According to the results of bacteriological studies it was found that the total contamination of 36 samples of 5 types of feed (SK-1, SK-3, SK-5, SK-6, and SK-31) is within acceptable limits and corresponds to veterinary and sanitary quality (Table 1).

However, it should be noted that all 5 studied samples of feed "SK-1" for pregnant sows contained an association of pathogenic microorganisms, namely Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria sica, Candida albicans, Aspergillus niger in the form of continuous growth on nutrient media.

Actinobacillus pleuropneumonia microorganisms were cultured at 37.0±0.5 °C for 24 hours on MPA with the addition of 10% yeast extract. The culture of A. pleuropneumonia had the form of small, convex, round, with smooth edges and mucous consistency colonies with a diameter of 0.5-1.5 mm. When culturing actinobacilli in PMB with yeast extract, bacterial growth was observed in the form of uniform turbidity of the medium with the formation of a white precipitate at the bottom, which...
was easily broken by shaking; on 5% blood agar with the addition of 10% yeast extract the growth of small translucent colonies with a diameter of 0.2-1.5 mm with smooth edges surrounded by a transparent zone of β-hemolysis was observed. According to the biochemical properties of the bacterium *A. pleuropneumonia* catabolized D-glucose and fructose with the formation of acid, were positive for β-galactosidase and negative for methyl red and for the formation of indole. During microscopy of smears in the field of view we noted gram-negative, small short rods, located singly.

**Table 1. Microbiological content of pig feed (n=36)**

| Polluting substance                  | Maximum allowable content, CFU, norm | SK-3 | SK-5 | SK-6 | SK-31 | SK-1 |
|--------------------------------------|--------------------------------------|------|------|------|-------|------|
| Total bacterial contamination        | not more 5 × 10⁵                      | 3 × 10⁵ | 4 × 10⁵ | 2 × 10⁵ | 4 × 10⁵ | 34 × 10⁵ |
| Enterobacteria                       | not more 300                         | -    | -    | -    | -     | -    |
| Salmonella in 50 g                   | are not allowed                       | -    | -    | -    | -     | -    |
| Pathogenic strains *E. coli*         | are not allowed                       | -    | -    | -    | -     | -    |
| Sulfite-reducing clostridia in 1 g   | are not allowed                       | -    | -    | -    | -     | -    |
| Pathogenic yersinia in 50 g          | are not allowed                       | -    | -    | -    | -     | -    |
| Coagulase-positive *S. aureus*       | are not allowed                       | -    | -    | -    | -     | -    |

Isolates of *Pasteurella multocida* during cultivation on the PBM after 24 h at a temperature of 37.0±0.5 °C formed a uniform turbidity of the medium. At the bottom of the tube a precipitate of a mucous nature was formed, which when shaken rose in the form of a tape. The obtained isolated colonies were subcultured on a dense nutrient medium (MPA) to obtain a pure culture, and the growth of small grayish colonies of mucous consistency was observed. On Hotinger’s agar 24 h after cultivation, the appearance of round, convex, with a smooth, moist surface, smooth edges translucent colonies with a diameter of 1-3 mm was noted; on blood agar with the addition of 5% sheep blood hemolysis zone was absent. According to the biochemical properties bacterium *P. multocida* catabolized D-glucose and fructose with the formation of acid, were oxidase- and catalase-positive, reduced nitrate to nitrite, samples with methyl red and for the formation of acetoin (Voges-Proskauer), as well as for lysisin carboxylase, arginine dihydrolase and gelatinase were negative. Microscopy of smears from colonies of cultures revealed gram-negative cells, coccoid-ovoid and ovoid forms with a pronounced bipolarity. There was no mobility.

Cultures of *Neisseria sicca* after 24 h of cultivation at a temperature of 37.0±0.5 °C on the MPA formed small transparent colonies with a blue tinge; turbidity was registered in the PMB; small transparent colonies resembling dewdrops appeared on the serum agar. Colonies of diplococci cultures on blood agar were small, round, transparent, surrounded by an α-zone of hemolysis (green zone). According to the biochemical properties of the bacterium *Neisseria sicca* oxidase- and catalase-positive, formed carbonic anhydrase, reduced nitrite. During microscopy, gram-negative cocci in the form of pairs 0.6-0.8 μm in size were observed in the field of view of the microscope.

Isolates of *Clostridium perfringens* on Kitt-Tarozzi medium after 24 h of cultivation at a temperature of 37.0±0.5°C formed turbidity with gas formation. Round smooth grayish colonies were recorded on 5% blood agar, which gradually turned green and were surrounded by a β-zone of hemolysis. Blackening and rupture of agar were recorded on Wilson-Blair medium after 1 h of cultivation. Clostridia fermented lactose, glucose, sucrose, maltose to form acid and gas, slowly diluted gelatin, curdled litmus milk with the formation of a brick-colored clot and complete enlightenment of whey. Reduced nitrates to nitrites, indole was not formed. Polymorphic rod-shaped gram-positive bacteria were observed in the field of view of the microscope. Spores were oval, centrally or subterminally located, immobile.

The *Candida albicans* culture on wort agar at 22.0±0.5 °C after 3 days formed round, shiny, flat or convex, colonies with smooth edges. Colonies on glucose-peptone medium were white-cream with a dull sheen, smooth, moist. On microscopy, *Candida albicans* looked like rounded or slightly elongated budding cells. To determine the species of *Candida albicans*, a test for the formation of germinal (embryonic tubes) was performed. A colony of a 24-hour yeast culture was added to a test tube with 0.5 ml of sterile sheep serum and kept for 3 hours at 37.0±0.5 °C. After 3 hours of incubation of the samples in a thermostat at 37.0±0.5 °C, the contents of the tube were placed on a glass slide and examined under a microscope. In the field of view of the microscope, a characteristic absence of cell narrowing at the base of the germ tubes was observed, where it is formed from the mother cell, which is characteristic of *Candida albicans* (Saigal et al., 2011). These microorganisms fermented glucose, maltose, sucrose, galactose, did not ferment lactose and raffinose.

Microscopic fungi *Aspergillus niger* have been identified by morphological and biochemical properties (McClenney, 2005). Thus, *Aspergillus niger* on wort agar at a temperature of 37.0±0.5°C after 3 days formed a branched, multinucleate mycelium of black color. On Chapek’s agar, *A. niger* agar colonies under microscopy had the appearance of filamentous fungi, the hyphae of which resembled the structure of a plant and formed smooth and colorless conidiophores with conidial heads (beads) with black or dark brown spores. On glucose agar, *A. niger* isolates isolated citric acid and according to the test for the decomposition of starch to glucose contained the enzyme glucoamylase (Geiser et al., 2007).

In the study of blood of sows fed with this feed, 20 of the 60 samples contained the same bacterial associations as the feed samples.

Isolated from feed and blood isolates of bacteria *Actinobacillus leupropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria sicca* had the ability to form biofilms in vitro, ie were biofilm-forming. For the ability to form biofilms, the
association of bacteria was cultured in 96-well polystyrene plates (O’Toole, 1998). After culturing, planktonic cells were removed from the wells by washing with phosphate-buffered saline. A 0.1% solution of gentian violet was added to the wells for staining. After incubation for some time, the dye was decanted, washed with distilled water and extracted with ethanol. Optical density was measured on a microplate spectrophotometer, evaluating its ability to biofilm formation. The optical density of the associated microflora reached > 4 optical units, which is the highest level of density (Stepanovic et al., 2007).

Biofilm-forming isolates of bacteria *Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria sicca, Candida albicans, Aspergillus niger* from feed and blood showed multidrug resistance, up to 30 antimicrobials (synthetic penicillins, cephalosporins, aminoglycosides, fluoroquinolones, tetracyclines, combined drugs); only the combined drug bromodox (bromhexine + doxycycline) was clinically effective (Table 2).

**Table 2. Sensitivity of biofilm-forming bacteria to antimicrobial drugs**

| Antimicrobial drug | Content in disk, mcg | Diameter of zones of growth inhibition, mm | Resistant (R) | Moderately sensitive (M) | Sensitive (S) |
|--------------------|----------------------|------------------------------------------|---------------|--------------------------|---------------|
| Synthetic penicillins |                      |                                          |               |                          |               |
| Amoxiclav          | 10                   | ≤13                                      | –             | –                        | –             |
| Amoxicillin        | 20                   | ≤13                                      | –             | –                        | –             |
| Oxacillin          | 1                    | ≤11                                      | –             | –                        | –             |
| Ampicillin         | 10                   | ≤11                                      | –             | –                        | –             |
| Cephalosporins     |                      |                                          |               |                          |               |
| Ceftiofur          | 30                   | ≤14                                      | –             | –                        | –             |
| Cefuroxime         | 30                   | ≤14                                      | –             | –                        | –             |
| Cefoxitin          | 30                   | ≤14                                      | –             | –                        | –             |
| Cefipim            | 30                   | ≤14                                      | –             | –                        | –             |
| Tetracyclines      |                      |                                          |               |                          |               |
| Doxycycline        | 30                   | ≤12                                      | –             | –                        | –             |
| Oxytetracycline    | 30                   | ≤14                                      | –             | –                        | –             |
| Remacycline        | 30                   | ≤14                                      | –             | –                        | –             |
| Aminoglycosides    |                      |                                          |               |                          |               |
| Kanamycin          | 30                   | ≤13                                      | –             | –                        | –             |
| Gentamicin         | 10                   | ≤12                                      | –             | –                        | –             |
| Spectinomycin      | 30                   | ≤12                                      | –             | –                        | –             |
| Neomycin           | 30                   | ≤12                                      | –             | –                        | –             |
| Fluoroquinolones   |                      |                                          |               |                          |               |
| Ofloxacin          | 5                    | ≤12                                      | –             | –                        | –             |
| Marbofloxacin      | 5                    | ≤14                                      | –             | –                        | –             |
| Gatifloxacin       | 5                    | ≤14                                      | –             | –                        | –             |
| Levofloxacin       | 5                    | ≤13                                      | –             | –                        | –             |
| Combined drugs     |                      |                                          |               |                          |               |
| Gentamicin sulfate + ofloxacin | 30 | ≤12 | – | – |
| Colistin sulfate + doxycycline | 30 | ≤10 | – | – |
| Colistin sulfate + thiamulin | 30 | ≤8 | – | – |
| Colistin sulfate + enrofloxacin | 30 | ≤8 | – | – |
| Tilmicosin + bromhexine | 30 | ≤10 | – | – |
| Bromhexine + doxycycline | 30 | ≤7 | – | ≥16 |
| Trimethoprim + sulfadimesine | 30 | ≤6 | – | – |
| Colistin sulfate + levofloxacin | 30 | ≤7 | – | – |
| Tylosin + doxycycline | 30 | ≤10 | – | – |
| Colistin sulfate + amoxicillin | 30 | ≤12 | – | – |
| Lincomycin + Spectinomycin | 30 | ≤12 | – | – |
Antibiotic resistance of microorganisms in biofilms is due to the ability of bacteria to accumulate in the matrix extracellular enzymes that destroy antimicrobial substances (Stacy et al., 2014).

No deaths were recorded during infection of white mice with biofilm-forming isolates of *Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria* spp., isolated from compound feed. While biofilm-forming isolates of the same bacteria infected laboratory animals, up to 30% of mice died: that is, variants of biofilm-forming “feed bacteria” adapted to pigs showed weakly virulent properties. However, this indicates that biofilms that enter the body of animals with food undergo passage, acquire virulence properties and, as a consequence, cause complications with a chronic course and are antibiotic-resistant.

Since the grain for the studied batches of feed came from forage lands fertilized with manure from the examined pig complex, it can be assumed with high probability that the biofilms of bacteria *Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria* spp. can successfully overcome the modern technological chain “from the field - to the feeder” in the composition of manure, which fertilizes the forage lands. This may be the basis for the formation and rooting of stationary foci of infection. The next stage of research was to determine the antagonistic properties of prebiotic and probiotic drugs, i.e., their suitability for the destruction of biofilms of pathogenic bacteria in feed (Fig. 1).

After treatment of biofilms of microorganisms, the Prebiotic agent (Fig. 1b) showed bactericidal activity against *P. multocida, Neisseria* spp., *Candida albicans*, but did not act on *A. pleuropneumonia*. Probiotic agent No 2 (concentrate of probiotic bacteria and fungi for food disinfection) on the contrary destroyed *A. pleuropneumonia*, and bacteria *P. multocida, Neisseria* spp.; *Candida albicans* showed resistance to its action (Fig. 1c). At that time, Probiotic agent No 1 (Bischofite-based composition with probiotics) destroyed the bacterial biofilm, but did not act on *P. multocida* (Fig. 1d). Thus, it is proved that each of the presented drugs acts on a certain type of bacteria, but they all destroy the fungi *Candida albicans*, which create the framework and conditions for the creation of biofilms with Pasteurella, neisseria and actinobacillus.

Microorganisms in biofilms show an altered phenotype in terms of growth rate and gene transcription compared to freely existing forms of the same organisms. Subsequent studies have shown that the phenotype of the biofilm can be described using genes of expressed cells (Donlan & Costerton, 2002). Biofilms are clinically important in both human and veterinary medicine, and have the ability to form on both objects and living tissue (Paterson, 2017). The ability to form biofilms is now considered a universal attribute of microorganisms (Jacques et al., 2010). Typically, biofilms are found in chronic diseases that oppose the host's immune response and antibiotic treatment (Hall-Stoodley & Stoodley,
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2009). Thus, biofilm formation can be considered an important virulence factor (Landini et al., 2010). Bacteria in biofilms are able to protect against stress, including resistance to antibiotics, disinfectants and humoral and cellular parts of the animal’s immune system (Bufjold & MacInnes, 2015).

The high ability of *Pseudomonas aeruginosa* to form biofilms has been proven, but no significant differences were found between isolates from animals and from humans (Miliwojievic et al., 2018). One of the most important food pathogens is *Campylobacter jejuni*, which also has the ability to form a biofilm on stainless steel, glass or polyvinyl chloride (Moe et al., 2010). Other studies suggest that *A. pleuropneumoniae* has the ability to form biofilms under appropriate growth conditions, and the transition from a biofilm-positive to a biofilm-negative phenotype is reversible (Labrie et al., 2010).

An important feature of bacterial biofilms is high resistance to antibacterial drugs. Antibiotic resistance of such a biofilm is due to the fact that it includes mechanisms (biofilm matrix) that prevent the penetration of antibiotics into the deep layers of the biofilm and disrupt direct contact with bacterial cells and usually show resistance to many antibiotics from different groups (Ruzicka et al., 2007; Stacy et al., 2014). Biofilm antibiotic resistance is likely to manifest as a combination of innate and induced mechanisms (Anderson & O’Toole, 2008). The problem of antibiotic resistance of pathogenic microorganisms is acute in other areas of animal husbandry (Hadzeyvych et al., 2019; Kasiyanenko et al., 2020). The mechanisms of resistance of biofilm bacteria to antibacterial agents allow them to remain viable at concentrations of antibiotics tens and hundreds of times higher than therapeutic doses that inhibit planktonic forms (Mah, 2012; Sager et al., 2015; Ramírez-Castillo et al., 2018).

Focused study of the role of biofilm-forming microorganisms in epizootiology and infectious pathology of animals began only a few years ago, but it is now clear the significant difference in the properties of infectious agents between their biofilm and planktonic life forms. The above results indicate a high risk for pig farming of the presence of even trace amounts (10-12 × 10^18 CFU/g) of avirulent variants of pathogenic bacterial species in feed. They also point to the danger of insufficiently decontaminated manure, which together with microbial biofilms is traditionally exported to forage lands, as a significant risk factor for the formation of foci of enzootic diseases in pig farming. Similar problems have been identified in fish farming, which is associated with the release of a number of pathogenic microorganisms from the ponds (Nazarenko et al., 2020).

Involvement of microbial biofilms in clinical veterinary medicine provides a change in approaches to the treatment of diseases associated with biofilms, as traditional antibiotic therapy does not solve this problem. The treatment of infections associated with biofilms is very complex. This primarily applies to chronic infections. As our results show, we can increase the effectiveness of their treatment with the use of the latest probiotics, which combine high antagonistic activity and resistance to adverse environmental factors of spore probiotic bacteria with bactericidal and mycoidal properties of antiseptics and detergents.

In the biofilms, bacteria acquire qualitatively new properties compared to microorganisms in planktonic form (Romling & Balsalobre, 2012; Tremblay et al., 2013; Sanchez et al., 2013). To increase the effectiveness of countering biofilms of pathogenic microorganisms in feed and animals, it is necessary to determine not only the bactericidal properties of antimicrobials, but also their ability to inhibit bacterial adhesion, penetrate biofilms, inhibit their formation or contribute to extracellular matrix disorganization (Schlegelová et al., 2008; Romanko et al., 2016; Roy et al., 2018). In addition, the effectiveness of some plant components as antimicrobial compounds against bacterial biofilms has been proven (Durig et al., 2010). Without adequate and effective diagnostic and treatment protocols for biofilm infections in animals, their impact on animal health will remain a serious problem (Richards & Melander, 2009; Gardner et al., 2011).

Along with bacterial contamination of livestock facilities, the current problem is the spread of exogenous forms of animal helminths in the environment (Paily et al., 2018b, 2019), as well as a number of ectoparasites (Paily et al., 2018a, 2018c). Only a comprehensive approach to the planning and organization of anti-epizootic measures will increase the culture of the livestock industry and will allow to obtain quality and safe products (Zavgorodniy et al., 2013; Paily et al., 2020). Based on this, the immediate task for us is to study the effectiveness of the latest probiotics for disinfection of the feed chain in pig breeding from pathogenic microorganisms.

**Conclusions**

According to the results of the conducted researches it was established that 5 samples of compound feed for pregnant sows contained the association of biofilm-forming variants of microorganisms Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria spp., Candida albicans, Aspergillus niger. The blood of sows fed with microbially contaminated feed “SK-1” contained the same bacterial associations as feed samples.

Biofilm-forming variants of *Actinobacillus pleuropneumonia*, *Pasteurella multocida*, *Clostridium perfringens*, *Neisseria* spp., *Candida albicans*, *Aspergillus niger* showed multidrug resistance to 30 antimicrobial drugs (synthetic penicillins, cephalosporins, aminoglycosides, fluoroquinolones, tetracyclines, combined drugs). It was found that 30% of isolates of microorganisms isolated from the blood of sows, in contrast to isolates of the same species of bacteria induced from feed “SK-1”, were pathogenic for laboratory mice.

Complex ‘Probiotic agent № 1’ (composition based on Bischofite with probiotics) completely destroys the microbial biofilm in the composition of *Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria* spp., *Candida albicans, Aspergillus niger*, however, it does not have a bactericidal effect on the planktonic form of *P. multocida*.

**References**

Abdullahi, U. F., Igwenagu, E., Mu’azu, A., Aliyu, S., & Umar, M. I. (2016). Intrigues of biofilm: A perspective in veterinary medicine. Veterinary world, 9(1), 12-18. doi: 10.14202/vetworld.2016.12-18

Ali, A., Danbappa, R., Alhassan, K. A., & Shah, M. M. (2018). Isolation and identification of microbial contaminants associated with commercial poultry feeds. Journal of Applied and Advanced Research, 3(5), 142-147. doi: 10.21839/jaar.2018.v3i5.231
Anderson, G. G., & O'Toole, G. A. (2008). Innate and induced resistance mechanisms of bacterial biofilms. Current topics in microbiology and immunology, 322, 85-105. doi: 10.1007/978-3-540-75418-3_5

Bintsis, T. (2018). Microbial pollution and food safety. AIMS Microbiology, 4(3), 377-396. doi: 10.3934/microbiol.2018.3.377

Bujold, A. R., & MacInnes, J. I. (2015). Identification of putative adhesins of Actinobacillus suis and their homologues in other members of the family Pasteurellaceae. BMC Research Notes, 8, 675. doi: 10.1186/s13104-015-1659-x

Clutterbuck, A. L., Woods, E. J., Knottenbelt, D. C., Clegg, P. D., Cochrane, C. A., & Percival, S. L. (2007). Biofilms and their relevance to veterinary medicine. Veterinary microbiology, 121(1-2), 1-17. doi: 10.1016/j.vetmic.2006.12.029

Crump, J. A., Griffin, P. M., & Angulo, F. J. (2002). Bacterial Contamination of Animal Feed and Its Relationship to Human Foodborne Illness. Clinical Infectious Diseases, 35(7), 859-865. doi: 10.1086/324885

Donlan, R. M., & Costerton, J. W. (2002). Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms. Clinical Microbiology Reviews, 15(2), 167-193. doi: 10.1128/CMR.15.2.167-193.2002

Dünig, A., Kouskoumvrekai, I., Vejborg, R. M., & Klemm, P. (2010). Chemoinformatics-assisted development of new anti-biofilm compounds. Applied microbiology and biotechnology, 87(1), 309-317. doi: 10.1007/s00253-010-2471-0

Flemming, H. C., & Wingender, J. (2010). The Biofilm Matrix. Nature Reviews Microbiology, 8, 623-633. doi: 10.1038/nrmicro2415

Gardner, A. J., Percival, S. L., & Cochrane, C. A. (2011). Biofilms and Role to Infection and Disease in Veterinary Medicine. From book Biofilms and Veterinary Medicine, 111-128. doi: 10.1007/978-3-642-21289-5_4

Geiser, D. M., Klich, M. A., Frisvad, J. C., Peterson, S. W., Varga, J., & Samson, R. A. (2007). The current status of species recognition and identification in Aspergillus. Studies in Mycology, 59, 1-10. doi: 10.3114/sim.2007.59.01

Haddzeychv, O. V., Paliy, A. P., Kinash, O. V., Petrov, R. V., & Paliy, A. P. (2019). Antibiotic resistance of microorganisms isolated from milk. World of Medicine and Biology, 3(69), 245-250. doi: 10.26724/2079-8334-2013-6-3-69-245-250

Hall-Stoodley, L., & Stoodley, P. (2009). Evolving concepts in biofilm infections. Cellular microbiology, 11(7), 1034-1043. doi: 10.1111/j.1462-5822.2009.01323.x

Jacques, M., Aragon, V., & Tremblay, Y. D. N. (2010). Biofilm formation in bacterial pathogens of veterinary importance. Animal Health Research Reviews, 11(2), 97-121. doi: 10.1016/S1466252310000149

Kasianenko, O. I., Kasianenko, S. M., Paliy, A. P., Petrov, R. V., Kambur, M. D., Zamaziy, A. A., Livoshchenko, L. P., Livoshchenko, Ye. M., Nazarenko, S. M., Klishchova, Zh. E., & Paliy, A. P. (2020). Application of mannan oligosaccharides (Altech Inc.) in waterfowl: optimal dose and effectiveness. Ukrainian Journal of Ecology, 10(3), 63-68. doi: 10.15421/2020_134

Labrie, J., Pelletier-Jacques, G., Deslandes, V., Ramjeet, M., Auger, E., Nash, J. H., & Jacques, M. (2010). Effects of growth conditions on biofilm formation by Actinobacillus pleuropneumoniae. Veterinary research, 41(1), 3. doi: 10.1051/vetres/2009051

Landini, P., Antoniani, D., Burgess, J. G., & Nijland, R. (2010). Molecular mechanisms of compounds affecting bacterial biofilm formation and dispersal. Applied microbiology and biotechnology, 86(3), 813-823. doi: 10.1007/s00253-010-2468-8

Lasa, I., Del Pozo, J. L., Penadés, J. R., & Leiva, J. (2005). Bacterial biofilms and infection. Anales del Sistema Sanitario de Navarra, 28(2), 163-175. doi: 10.4321/s1317-66722005000300002

Machado-Moreira, B., Richards, K., Brennan, F., Abram, F., & Burgess, C. M. (2019). Microbial Contamination of Fresh Produce: What, Where, and How? Comprehensive Reviews in Food Science and Food Safety, 18(6), 1727-1750. doi: 10.1111/1541-4337.12487

Maciorowski, K. G., Herrera, P., Jones, F. T., Pillai, S. D., & Ricke, S. C. (2007). Effects on poultry and livestock of feed contamination with bacteria and fungi. Animal Feed Science and Technology, 133(1-2), 109-136. doi: 10.1016/j.anifeedsci.2006.08.006

Mahami, T., Tobgy-Tetteh, W., Kottoh, D. I., Amoakoah-Twum, L., Gasu, E., Annan, S. N. Y., Larbi, D., Adjei, I., & Adu-Gyamfi, A. (2019). Microbial Food Safety Risk to Humans Associated with Poultry Feed: The Role of Irradiation. International Journal of Food Science, 2019, ID 6915736. doi: 10.1080/13693780500052222

Mah, T. F. (2012). Biofilm-specific antibiotic resistance. Future microbiology, 7(9), 1061-1072. doi: 10.2217/fmb.12.76

Milivojevic, D., Šumonja, N., Medić, S., Pavic, A., Moric, I., Vasiljevic, B., Senerovic, L., & Nikodinovic-Runic, J. (2018). Biofilm-forming ability and infection potential of Pseudomonas aeruginosa strains isolated from animals and humans. Pathogens and Disease, 76(4), fty041. doi: 10.1093/femsdp/fty041

Moe, K. K., Mimura, J., Ohnishi, T., Wake, T., Yamazaki, W., Nakai, M., & Misawa, N. (2010). The mode of biofilm formation on smooth surfaces by Campylobacter jejuni. The Journal of veterinary medical science, 72(4), 411-416. doi: 10.1292/jvms.09-0339

Nazarenko, S. M., Paliy, A. P., Berezovskiy, A. V., Fotin, A. I., Fotin, O. V., Petrov, R. V., Kasianenko, O. I., Lazorenko, L. N., Negreba, J. V., Paliy, A. P., & Rebenko, H. I. (2020). Improving the sanitary condition of pond bed by forage grass cultivation. Ukrainian Journal of Ecology, 10(2), 368-374. doi: 10.15421/2020_111

O'Toole, G. A., & Kolter, R. (1998). Initiation of biofilm formation in Pseudomonas fluorescens WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. Molecular microbiology, 28(3), 449-461. doi: 10.1046/j.1365-2958.1998.00797.x

Paliy, A. P., Masheyk, A. M., Sumakova, N. V., & Paliy, A. P. (2018a). Distribution of poultry ectoparasites in industrial farms, farms, and private plots with different rearing technologies. Biosystems Diversity, 26(2), 153-159. doi: 10.15421/011824

Paliy, A. P., Sumakova, N. V., Masheyk, A. M., Petrov, R. V., Paliy, A. P., & Ishchenko, K. V. (2018b). Contamination of animal-keeping premises with eggs of parasitic worms. Biosystems Diversity, 26(4), 327-333. doi: 10.15421/011849

Paliy, A. P., Sumakova, N. V., Paliy, A. P., & Ishchenko, K. V. (2018c). Biological control of house fly. Ukrainian Journal of Ecology, 8(2), 230-234. doi: 10.15421/2018_332
Paliy, A. P., Zavgorodnyi, A. I., Stegniy, B. T., & Paliy, A. P. (2020). Scientific and methodological grounds for controlling the development and use of disinfectants. Monograph. Kharkiv: «Miskdruk», 318. ISBN: 978-617-619-237-4. (in Ukrainian)

Paliy, A., Sumakova, N., Petrov, R., Shkromada, O., Ulko, L., & Paliy, A. (2019). Contamination of urbanized territories with eggs of helminths of animals. Biosystems Diversity, 27(2), P. 118-124. doi: 10.15421/011916

Paterson, S. (2017). Biofilms: their importance in veterinary medicine. Companion Animal, 22(11), 659-668. doi: 10.1298/coan.2017.22.11.659

Pereira, C. S., Cunha, S. C., & Fernandes, J. O. (2019). Prevalent Mycotoxins in Animal Feed: Occurrence and Analytical Methods. Toxins, 11(5), 290. doi: 10.3390/toxins11050290

Pereyra, C. M., Cavagneri, L. R., Chiachiera, S. M., & Dalceo, A. M. (2010). Fungi and Mycotoxins in Feed Intended for Sows at Different Reproductive Stages in Argentina. Veterinary Medicine International, 2010, ID 569108. doi: 10.4061/2010/569108

Ramirez-Castillo, F. Y., Loera-Muro, A., Vargas-Padilla, N. D., Moreno-Flores, A. C., Avelar-González, F. J., Harel, J., Jacques, M., Oropesa, R., Barajas-García, C. C., & Guerrero-Barrera, A. L. (2018). Incorporation of Actinobacillus pleuropneumoniae in Preformed Biofilms by Escherichia coli Isolated From Drinking Water of Swine Farms. Frontiers in veterinary science, 5, 184. doi: 10.3389/fvets.2018.00184

Richards, J. J., & Melander, C. (2009). Controlling bacterial biofilms. Chembiochem, 10(14), 2287-2294. doi: 10.1002/cbic.200900317

Romanko, M., Yaroshenko, M., Orochchenko, A., Kutsan, A., Paliy, A., & Dubin, R. (2016). Study of antymycotic properties of Ag and Cu nanoparticles and their compositions in experiments on models of test-culture Aspergillus fumigatus. Pasze Przemysłowe, 3/4, 87-91.

Romling, U., & Balsalobre, C. (2012). Biofilm infections, their resilience to therapy and innovative treatment strategies. Journal of Internal Medicine, 272, 541-562. doi: 10.1111/joim.12004

Roy, R., Tiwari, M., Donelli, G., & Tiwari, V. (2018). Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. Virulence, 9(1), 522-554. doi: 10.1080/21505594.2017.1313372

Růžička, F., Holá, V., Votava, M., & Tejkalová, R. (2007). Importance of biofilm in Candida parapsilosis and evaluation of its susceptibility to antifungal agents by colorimetric method. Folia microbiologica, 52(3), 209-214. doi: 10.1007/BF02931300

Sager, M., Benten, W. P., Engelhardt, E., Gougoula, C., & Benga, L. (2015). Characterization of Biofilm Formation in [Pasteurella] pneumotropica and [Actinobacillus] muris Isolates of Mouse Origin. PLoS One, 10(10), e0138778. doi: 10.1371/journal.pone.0138778

Saigal, S., Bhargava, A., Mehra, S. K., Dawkala, F. (2011). Identification of Candida albicans by using different culture media and its association in potentially malignant and malignant lesions. Contemporary clinical dentistry, 2(3), 188-193. doi: 10.4103/0976-237X.86454

Sanchez, C. J., Mende, K., Beckius, M. L., Akers, K. S., Romano, D. R., Wenke, J. C., & Murray, C. K. (2013). Biofilm formation by clinical isolates and the implications in chronic infections. BMC Infectious Diseases, 13, 47. doi: 10.1186/1471-2334-13-47

Schillaci, D., & Vitale, M. (2012). Biofilm Related to Animal Health, Zoonosis and Food Transmitted Diseases: Alternative Targets for Antimicrobial Strategy? Journal of Microbial & Biochemical Technology, 4(4), 7-10. doi: 0.4172/1948-5948.1000e108

Schlegelová, J., Babáková, V., Holasová, M., & Dendis, M. (2008). The biofilm-positive Staphylococcus epidermidis isolates in raw materials, foodstuffs and on contact surfaces in processing plants. Folia microbiologica, 53(6), 500-504. doi: 10.1007/s12223-008-0078-y

Shirokikh, I. G., Kozlova, L. M., Shirokikh, A. A., Popov, F. A., & Tovstik, E. V. (2017). Effects of tillage technologies and application of biopreparations on micromycetes in the rhizosphere and rhizoplane of spring wheat. Eurasian Soil Science, 50, 826-831. doi: 10.1134/S1064229317070110

Stacy, A., Everett, J., Jorth, P., Trivedi, U., Rumbaugh, K. P., & Whiteley, M. (2014). Bacterial fight-and-flight responses enhance virulence in a polymicrobial infection. Proceedings of the National Academy of Sciences, 111(21), 7819-7824. doi: 10.1073/pnas.1400586111

Stepanovic, S., Vukovic, D., Hola, V., di Bonaventura, G., Djukić, S., & Ćirković, I. (2007). Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for 42 assessment of biofilm production by Staphylococci. APMIS, 115(8), 891-899. doi: 10.1111/j.1600-0463.2007.apm_630.x

Tamang, J. P., Shin, D. H., Jung, S. J., & Chae, S. W. (2016). Functional Properties of Microorganisms in Fermented Foods. Frontiers in microbiology, 7, 578. doi: 10.3389/fmicb.2016.00578

Tremblay, Y. D., Lévesque, C., Segers, R. P., & Jacques, M. (2013). Method to grow Actinobacillus pleuropneumoniae biofilm on a biotic surface. BMC Veterinary Research, 9, 213. doi: 10.1186/1746-6148-9-213

Van Houdt, R., & Michiels, C. W. (2010). Biofilm formation and the food industry, a focus on the bacterial outer surface. Journal of applied microbiology, 109(4), 1117-1131. doi: 10.1111/j.1365-2672.2010.04756.x

Zavgorodnyi, A. I., Stegniy, B. T., Paliy, A. P., Gorjeev, V. M., & Smirnov, A. M. (2013). Scientific and practical aspects of disinfection in veterinary medicine. Kharkiv: FOP Brovin O.V. (in Ukrainian)

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