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PATHOGENICITY OF NON-PIGMENTED AND PIGMENTED ISOLATES OF *P. AERUGINOSA* DURING LONG-TERM STORAGE

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The aim: *to study the pathogenicity of non-pigmented P. aeruginosa isolates compared to those that synthesize pigment during long-term storage for broiler chickens.*

Materials and methods. *Bacteriological studies were carried out in accordance with generally accepted methods. Initially, P. aeruginosa and other bacterial microflora were isolated from poultry farms, pathological material from poultry. The type of microorganisms was identified using Bergey's identifier. The obtained isolates were examined by morphological characteristics (according to Gram), tincture, cultural, biochemical, pathogenic properties and sensitivity of selected cultures to antibiotics were studied. After the expiration of 3 years, the main biological properties of the isolates were monitored. To establish pathogenic properties, a bioassay was performed on white mice and one-week-old broiler chickens of the Cobb 500 cross (intraperitoneally by washout from daily agar culture at a dose of 0.2 cm^3, which corresponds to the previously established LD50).*

The results. *With parenteral infection by washout from agar daily culture of pigmentless isolates at a dose of LD50, chickens died within the first – second day, in this case 87.50 % of the cultures showed pathogenicity. Experimental parenteral infection with washings from a daily culture of pigmented isolates of P. aeruginosa at a dose of LD50 led to the death of one-week-old chickens within 24-48 hours, in this case 75.00 % of the isolates showed pathogenicity.*

Clinical and pathological signs of infection with pigmented and non-pigmented P. aeruginosa isolates were similar.

Conclusions. *A comparative analysis of cases of pathogenicity of non-pigmented and pigmented isolates of P. aeruginosa on one-week-old broiler chickens of the Cobb-500 cross was carried out. In the experiment, we found that among the non-pigmented isolates, compared to the pigmented pathogenic isolates, 12.5 % more were detected in chickens. This emphasizes the importance of differential diagnosis for pseudomonosis, because infection with non-pigmented strains often goes undiagnosed*

Keywords: *P. aeruginosa, poultry pseudomonosis, pyocyanin pigment, pathogenicity, LD50, broiler chickens, culture storage, strains, isolates*

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1. Introduction

The problem of diagnosis, control and prevention of bacterial infections is important in modern poultry farming. It becomes especially relevant for the use of highly productive poultry of domestic and foreign breeding in order to obtain maximum production. In such conditions, the adaptation capabilities of the poultry organism to technological, feed, medicinal, bacterial, environmental and other stress factors of modern industrial poultry farming are reduced. The significance of opportunistic microorganisms among the causative agents of poultry diseases, which mostly exist in various associations, reduce the resistance of poultry compared to monoinfections and negatively affect the immune status of the organism, is just increasing. Under these conditions, diagnosis and timely implementation of antiepizootic measures are complicated [1–3].

The causative agent is a universal pathogen for almost all animals, humans, and plants. *P. aeruginosa* often complicates various pathological processes of both infectious and non-infectious etiology. One of the differential-diagnostic signs, which is paid attention to during the microbiological diagnosis of blue-pus infection, is the ability of the microorganism to synthesize a water-soluble pigment – pyocyanin. This substance belongs to the phenazine compound, is produced by representatives of the genus *Pseudomonas* and has a wide spectrum of antibiotic activity, which is found against gram-positive, gram-negative microorganisms and fungi. Pyocyanin is one of the virulence factors. Atypical (non-pigmented) forms of the pathogen complicate the diagnosis, preventing the timely implementation of medical and anti-epidemic measures. *P. aeruginosa* is an extremely unpretentious mi-

croorganism with wide adaptive capabilities and the ability to quickly form resistance to antibiotics [2–4].

The aim of the research was to study the pathogenicity of non-pigmented *P. aeruginosa* isolates compared to those that synthesize pigment during long-term storage for broiler chickens.

2. Materials and methods

The research was carried out during 2019–2022 on the basis of the laboratories «Innovative technologies and safety and quality of livestock products» and «Veterinary pharmacy» of the department of microbiology, veterinary and sanitary examination, animal hygiene and quality and safety of livestock products of the Faculty of Veterinary Medicine of Sumy National Agrarian University.

Bacteriological studies were carried out in accordance with generally accepted methods. Initially, *P. aeruginosa* and other bacterial microflora were isolated from poultry farms, pathological material from poultry. The type of microorganisms was identified using Bergey's identifier. The obtained isolates were examined by morphological characteristics (according to Gram), tincture, cultural, biochemical, pathogenic properties and sensitivity of selected cultures to antibiotics were studied. The sensitivity of isolates to antibacterial drugs was determined by the method of diffusion in agar in accordance with the generally accepted methodology.

8 isolates of *P. aeruginosa* (isolated from pathological material from adult chickens, broiler chickens, waterfowl) were selected for long-term storage studies. All isolates showed characteristic properties (formation of pigments pyocyanin, pyoverdine on MPA (meat peptone agar), MPB (meat-peptone broth); the aromatic substance trimethylamine, which has a specific smell of jasmine or strawberry; oxidation of glucose and galactose, inertness to mannitol, sucrose and lactose). From each isolate, 3 transplants were made in parallel in 3 tubes with MPA (for the reliability of the results). Isolates were stored at MPA under rubber caps at a temperature of (4–5) °C for 3 years. During the storage period, no research, no reseeding was carried out, only the fact of pigment formation was recorded visually.

After a period of 3 years, monitoring of the basic biological properties of the isolates was carried out. We studied morphological influences (by Gram), cultural influences – on MPB, MPA and with the addition of 1 % glucose, the Endo medium. The first visit was carried out at MPB, MPA. They were fed 5 passages on MPB, MPA at +37 °C, then 5 passages on MPB, MPA with 1 % glucose. We also checked the production of crops to grow beyond +42 °C and +5 °C. The approval of the pigment was established visually after the fact of changing the color of MPB, MPA after 24–48 hours to green. For a more intensive development of the biological process, aeration of the MPB was carried out: a test tube with 1–2 stock broth cultures was intensively drained a dozen times. Fluorescent pigment pioverdin was detected by UV light (Wood's lamp).

To establish pathogenic influences, a biological test was carried out on white mice and one-week-old broiler chickens of the Cobb 500 cross (intraperitoneally by washout from daily agar culture at a dose of 0.2 cm^3, which corresponds to the previously established LD50).

3. Research results

Theoretical and practical foundations of understanding about opportunistic pathogens (conditionally pathogenic microorganisms) and the processes that they imply to become contagious are presented. Conditionally pathogenic microorganisms are a group of microorganisms of different taxonomic positions that often cause a pathological process beyond the sane minds. The term «opportunistic pathogens» was formulated according to current knowledge as a name that helps to group various types of everyday life behind the usual manifestations. The increased importance of opportunistic pathogens does not establish a clear difference between pathogenic and conditionally pathogenic microorganisms. By the way, pathogenicity for obligate pathogenic microorganisms and mental concepts. It also manifests itself in the case of singing minds (sufficient infective dose, responsiveness to the body, etc.). In this case, the biological host may also be species-friendly; for other species, this pathogen may be non-pathogenic.

For pathogenic microorganisms, pathogenicity is an attribute of the species that originated and became entrenched in the process of evolution [5, 6]. Parasitism for many mentally pathogenic microorganisms is a necessary mental saving for the species. Conditionally pathogenic microorganisms have a weak pathogenic potential, and for this they will manifest the necessary complex of minds, such as a decrease in the level of the cellular nonspecific support of the body, a change in the drying power of normal microflora. Therefore, opportunistic pathogens are usually found in the breeding group (young animals, especially those with a reduced immune status, after a period of prolonged stagnation of antimicrobial agents, etc.).

The authors consider the pathogenicity of conditionally pathogenic microorganisms as biological power and ecological relations. This is the interaction of three factors: the biology of the parasite (the presence and nature of the aggression factors), the mechanisms of protection of the body (natural resistance), the environmental factors that change and facilitate the implementation of pathogenic powers. It is important that the concept of «pathogenicity» for opportunistic pathogens is based on a complex of factors, including some biological characteristics of the species, others - factors of sensitivity or resistance of the organism, at the same time worn to the present day environment, has an ecological background [7, 8].

P. aeruginosa is a universal pathogen of almost all species of animals, humans, and plants. The presence of non-pigmented signs significantly complicates diagnosis, «masks» the etiological factor of the pathological process and causes their further spread.

In the process of research, we drew attention to the phenomenon of instability of the biological properties of *P. aeruginosa* isolates, which were isolated during an associated course with other bacterial pathogens and during long-term storage and numerous reseeding on artificial nutrient media. For example, the formation of the pigments pyocyanin and pyoverdine (fluorescein) appeared to varying degrees during storage. During long-term storage, the main cultural and biochemical properties were preserved, but the manifestation of pigment formation changed: during storage and reseeding, pigment formation was suppressed, and after

passages through a biological organism, it was resumed, then again after reseeding through artificial nutrient media, pigment formation was suppressed.

During the studies of the biological properties of *P. aeruginosa* strains, which we isolated with other bacterial pathogens, in the conditions of numerous interruptions and long -term storage, we drew attention to the fact that strains with oppressed pigmentation retained pathogenicity. During the intraperitoneally infection, all isolates had a pathogenic effect on white mice within 24 hours.

A comparative analysis of cases of pathogenicity of non-pigmented and pigmented isolates *P. aeruginosa* on one-week-old COBB-500 chickens was carried out. In the experiment, we found that among the non-pigmented isolates compared to the pigmented ones of pathogenic strains, more than 12.5 % were detected in chickens.

With parenteral infection by washout from the daily bacterial non-pigmented isolates at a dose of LD50 chickens died during the first-second day, in which case 87.50 % of the isolates were pathogenic (Table 1).

Experimental parenteral infection with washout from the daily bacterial culture of pigmented isolates of *P. aeruginosa* at a dose of LD50 led to the death of one-week-old chickens within 24–48 hours, in this case 75.00 % of the isolates showed pathogenicity (Table 2).

Table 1

Pathogenicity of *P. aeruginosa* non-pigmented isolate on broiler chickens

Isolates *P. aeruginosa*	Day of research, Number of heads											
	1		2		3		4		5		6	
	Get sick	Died	Get sick	Died	Get sick	Died	Get sick	Died	Get sick	Died	Get sick	Died
1	5	3	–	–	–	–	–	–	–	–	–	–
2	5	2	–	1	–	–	–	–	–	–	–	–
3	5	2	–	2	–	–	–	–	–	–	–	–
4	5	2	–	1	–	–	–	–	–	–	–	–
5	5	2	–	–	–	–	–	–	–	–	–	–
6	5	3	–	1	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–	–	–	–
8	5	2	–	1	–	–	–	–	–	–	–	–

Table 2

Pathogenicity of *P. aeruginosa* pigmented isolate on broiler chickens

Isolates *P. aeruginosa*	Day of research, Number of heads											
	1		2		3		4		5		6	
	Get sick	Died	Get sick	Died	Get sick	Died	Get sick	Died	Get sick	Died	Get sick	Died
1	5	2	–	–	–	–	–	–	–	–	–	–
2	5	2	–	1	–	–	–	–	–	–	–	–
3	4	1	1	1	–	–	–	–	–	–	–	–
4	5	2	–	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–	–	–	–
6	3	1	–	1	–	–	–	–	–	–	–	–
7	5	2	–	–	–	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–	–	–	–	–	–

Clinical and pathological signs of infection with pigmented and non-pigmented isolates of *P. aeruginosa* were similar. General depression, ruffledness of chickens, refusal of feed, diarrhea in some were established. Pathological catarrhal and hemorrhagic inflammation of the lungs, intestines, hemorrhages on the epicardium, on the surface of the liver, spleen, and muscles were found.

4. Discussion of research results

A number of factors have been identified in opportunistic microorganisms that, under certain conditions, can provide a pathogenic effect on the body: surface structures responsible for attachment to epithelial cells, various extracellular products, such as exotoxins and pathogenicity enzymes, endotoxins, hypothetical invasiveness factors, the ability to resist host defense mechanisms, as well as factors determining selective advantages (resistance to antibacterial drugs) [9, 10].

Pseudomonas aeruginosa (syn. P. aeruginosa, P. pyocyanea, Hac. aeruginosum) refers to opportunistic microorganisms, was first isolated by Gessard in 1882, according to Bergey's definition, bacteria of the genus *Pseudomonas* belong to the *Pseudomonadaceae* family and unite more than 145 species. Among a large number of species, *P. aeruginosa* is of particular importance in poultry pathology. The disease proceeds according to the type of acute or chronic toxic infection and is accompanied by high mortality, especially of young birds and embryos. Morbidity and mortality varies between (30–40) % and can reach 90 %. In our studies, we revealed the fact of preservation and pronounced pathogenicity in non-pigmented strains compared to pigmented isolates of P. *aeruginosa*.

Advantages and disadvantages of the obtained research results.

The advantage of this study is that the fact of preservation and even prevalence of pathogenicity in non-pigmented strains compared to pigmented isolates was established, which emphasizes the need to use differential media for pigment detection. The disadvantage is that in this case there is no possibility not to use animals in this research.

Limitations of the study. Limitation of the study is that most poultry farms in Ukraine are private, so there may be difficulties in access for such studies.

Prospects for further research. The purpose of further research is the comparative analysis of antibiotic resistance of non-pigmented and pigmented isolates.

5. Conclusions

Isolates of *P. aeruginosa*, obtained by association with bacterial pathogens, during long-term storage, are characterized by a high level of viability (cultures do not lose pathogenic, biochemical and enzymatic properties for three years on MPA under rubber covers at a temperature of 4–5 °C without reseeding), as well as the ability to suppress and restore pigment formation, which complicates diagnosis and promotes the spread of hidden forms of infection. A comparative analysis of cases of pathogenicity of non-pigmented and pigmented isolates of *P. aeruginosa* on one-week-old broiler chickens of the Cobb-500 cross was carried out. In the experiment, we found that among the non-pigmented isolates, compared to the pigmented pathogenic isolates, 12.5 % more were detected in chickens. This emphasizes the importance of differential diagnosis for pseudomonosis, because infection with non-pigmented strains often goes undiagnosed.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results, presented in this article.

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Data availability

Manuscript has no associated data.

Using artificial intelligence tools

The authors confirm that they did not use artificial intelligence technologies when creating the presented work.

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