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### Dynamics of changes in bacteriological and morphometric parameters at decreasing colonization resistance of bird intestine

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**Abstract.** Decrease in compensatory mechanisms of natural resistance due to changes in composition of evolutionarily formed microbiocenoses, increases risks of developing a syndrome of excessive growth of antibiotic-resistant microorganisms. The aim of the study was to analyze the dynamics of changes in bacteriological and morphofunctional parameters with a decrease in colonization resistance of the intestine of birds. To assess quantitative and species composition of microorganisms, the colonization index of bacteria isolated from the caecum contents of intestine of clinically healthy and sick birds was considered. The dynamics of changes in morphofunctional parameters during the dissemination of pathogenic bacteria into tissues and organs was studied using cytological and histochemical data. The number of microorganisms isolated from the caecum contents of intestine of sick birds significantly increased, the colonization index of lactose-positive bacteria was 0.247...0.283; lactose-negative bacteria — 0.657...0.730. With excessive bacterial contamination of ileocecal part of intestine and translocation of pathogens outside gastrointestinal tract, signs of dystrophy, necrosis and rejection of epithelial cells of mucous membrane of respiratory and digestive systems developed. The initiation, development and outcome of the infectious process are mediated by stability of homeostasis of susceptible macroorganisms and realization of pathogenic potential of isolates producing adhesive antigens, bacteriocins, hemolysins, and cytotoxins.

**Key words:** adhesive antigens, bacteriocins, hemolysins, cytotoxins, bacterial dissemination, dystrophy, necrosis

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## Introduction

When kept in confined areas, artificial microclimate and continuous poultry farming system cause the development and wide distribution of infectious diseases [1–5]. In poultry farms of various technological directions, in generalized infectious process, the dominance of etiologically significant bacteria *Escherichia coli* has been established — 50.7%, *Enterococcus faecalis* — 25.4%, *Proteus mirabilis* — 8.4% [6]. From the small intestine of chickens growb in peasant farms and private subsidiary farms, the incidence of isolates of *E. coli* is 100%, *Enterococcus faecalis* — 85.0%, *Proteus vulgaris* — 55.0%, *Pantoea agglomerans* — 25.0%, *Citrobacter freundii* — 15.0%, *Klebsiella pneumoniae* — 10.0% [7]. Decrease in mechanisms of mucociliary clearance and colonization resistance of mucous membrane leads to increased risks of developing excessive growth and translocation of biofilm-forming mycoorganisms increase [8—11]. To optimize the scheme of microbiological diagnostics, rational antibiotic therapy and vaccination, studies of pathogenetic mechanisms of excessive growth of microorganisms with a decrease in natural resistance of mucosa-associated lymphoid tissue, as an integrating component of homeostasis stability, are of diagnostic and prognostic significance.

The purpose of the study was to analyze the dynamics of changes in bacteriological and morpho-functional indicators with a decrease in colonization resistance of intestine of birds.

## Materials and methods

When assessing the quantitative and species composition of microorganisms, the colonization index of bacteria isolated from the contents of caecum of clinically healthy and sick Grade Maker turkeys was considered. To account for the number of microorganisms, the intestinal contents weighing 1.0 g were placed in a test tube and 9.0 cm<sup>3</sup> of 0.85% NaCl solution were added. From diagnostically significant dilutions, 0.1 ml of the test sample was applied to the surface of differential diagnostic media. Microorganisms were cultured at 37 ± 1.0 °C for 24 ± 1 h and 48 ± 1 h. For species identification, three colonies of microorganisms typical for the species were transferred to test tubes with slanted Meat Infusion Agar (MIA) and cultured at 37 ± 1.0 °C for 24 ± 1 h. Indication and

identification of microorganisms were carried out in accordance with the methodological recommendations “Isolation and identification of bacteria of gastrointestinal tract of animals” (11.05.2004, No. 13-5-02/1043)<sup>1</sup>.

The dynamics of changes in morphofunctional indicators during dissemination of pathogenic bacteria into tissues and organs of birds was studied using cytological and histochemical data [12—15]. The experiments were carried out in accordance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union dated September 22, 2010. The experimental data were processed by statistical analysis using the Student's reliability criterion; the results were considered reliable at  $p \leq 0.05$ .

## Results and discussion

Depending on duration and severity of the disease, reliable ( $p \leq 0.05$ ) differences in quantitative and species composition of microorganisms isolated from the contents of caecum of clinically healthy and sick birds were revealed. With excessive growth of microorganisms, colonization index of lactose-positive bacteria was 0.247...0.283; lactose-negative bacteria — 0.657...0.730 (table).

**The amount of bacteria, Ig CFU/g, on Endo Agar at 37 ± 1.0 °C, 24 ± 1 h**

Medium	The amount of bacteria, Ig CFU/g		
	Control	Experiment	Colonization index *, %
Endo Agar, lactose “+”	1.70 ± 0.02–2.18 ± 0.03	6.0 ± 0.05–8.81 ± 0.08	0.247...0.283
Endo Agar, lactose “-”	5.04 ± 0.05–5.23 ± 0.05	6.90 ± 0.05–7.95 ± 0.08	0.657...0.730

\* Proportion of microorganisms, Ig CFU/g, in intestinal contents from clinically healthy birds (control) and from birds with diseases (experimental).

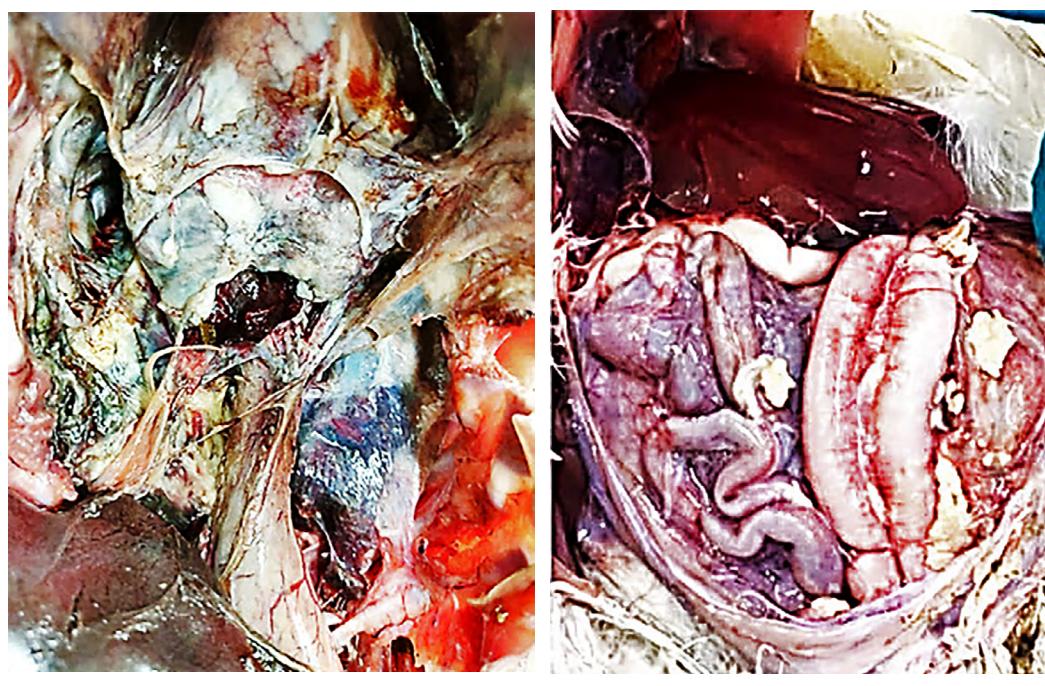
Source: compiled by E.M. Lenchenko, V.V. Ponomarev.

After identifying microorganisms isolated from caecum contents and pathological material of birds, the dominance of gram-negative, facultatively anaerobic, oxidase-negative, catalase-positive isolates was established. The main differential features of the isolates: *Escherichia* bacteria form indole, do not utilize sodium citrate and malonate, do not produce urease and phenylalanine deaminase. *Proteus* bacteria form hydrogen sulfide, urease, reduce nitrates, hydrolyze gelatin, ferment glucose, the reaction with methyl red is positive, deaminate phenylalanine, do not decarboxylate lysine, differ in the ability to utilize sodium citrate. *Klebsiella* bacteria utilize glucose, sodium citrate, produce acetyl methyl carbinol, ferment inositol, hydrolyze urea, do not form indole and

<sup>1</sup> Methodological recommendations «Isolation and identification of bacteria of gastrointestinal tract of animals. URL: <https://files.stroyinf.ru/Index2/1/4293723/4293723844.htm> (accessed: 12.09.2024).

hydrogen sulfide. *Serratia* bacteria do not form indole, do not ferment lactose, form lysine decarboxylase and ornithine decarboxylase. *Escherichia coli* isolates — 20 (100.0%) and *Proteus mirabilis* — 20 (100.0%) were identified from the cecum contents and pathological material of sick birds (n = 20). The incidence of *Citrobacter diversus* isolates was 11 (55.0%); *Serratia marcescens* — 10 (50.0%); *Klebsiella pneumoniae* — 1 (5.0%). The isolates produced adhesive antigens — 11.1%, α-, β-hemolysins — 28.1%, bacteriocins — 23.4%; cytotoxins — 21.4%. Due to the production of extended-spectrum β-lactamases, which determine the tendency of increasing multiple drug resistance, 56.6% of the isolates were resistant to semisynthetic penicillins and third-generation cephalosporins.

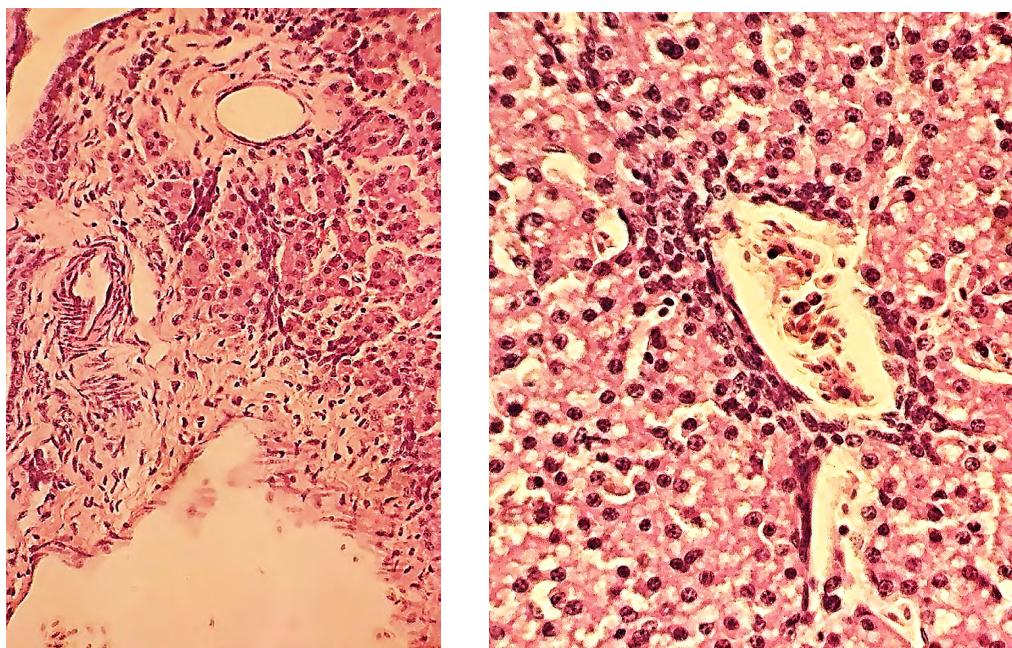
The etiological significance of the isolates *E. coli*, *P. mirabilis*, *C. diversus* was established in local and systemic pathology of 7 (35.0%) birds; *E. coli*, *P. mirabilis*, *S. marcescens* — 6 (30.0%) birds; *E. coli*, *P. mirabilis*, *S. marcescens*, *C. diversus* — 4 (20.0%) birds; *E. coli*, *P. mirabilis* — 2 (10.0%) birds; *E. coli*, *P. mirabilis*, *K. pneumoniae* — 1 (5.0%) bird. Depending on duration of exposure and degree of pathogenicity of the isolates, mucous membranes were cyanotic, ruffled feathers, dry skin, uneven and sharp bloating of the stomach and intestines, accumulation of hemorrhagic exudate, and multiple hemorrhages were detected (Fig. 1).



**Fig. 1.** Pathoanatomical signs in bacterial dissemination:  
a — *E. coli*, *P. mirabilis*, *S. marcescens*, *C. diversus*; b — *E. coli*, *P. mirabilis*, *K. pneumoniae*

Source: taken by E.M. Lenchenko, V.V. Ponomarev.

With an increase in number and pathogenic potential of isolates, the most frequently detected signs were hemorrhagic diathesis, catarrhal-hemorrhagic aerosacculitis, hemorrhagic enteritis, serous-fibrinous polyserositis, hemorrhagic splenitis. Reliably significant changes in morphometric parameters ( $p \leq 0.05$ ) of mucociliary clearance and colonization resistance were established when indicating gram-negative bacteria in smears-imprints of liver, kidneys, spleen. Excessive bacterial contamination of ileocecal section of intestine and translocation of pathogens beyond the gastrointestinal tract were accompanied by the development of dystrophy, necrosis and rejection of epithelial cells of mucous membrane of respiratory and digestive systems. The alkaline phosphatase activity of enterocytes of mucous membrane of villi in small intestine of sick birds decreased by 1.5 times compared to such indices of clinically healthy individuals. Along with congestion, plethora of vessels of portal tracts, dilation, and emptying of central vein and interlobular vessels were observed. Histiocytes and dust-like accumulations of hemosiderin pigment were detected in the lumen of intralobular sinusoidal capillaries. Disruption of the beam structure of lobules and polymorphism of hepatocytes were detected. Proliferative reactions of reticuloendothelial system were accompanied by perivascular infiltration of histiocytes and destructive processes of parenchymatous cells (Fig. 2).



**Fig. 2.** Turkey liver with bacterial dissemination *E. coli*, *P. mirabilis*, *S. marcescens*, *C. diversus*.  
Hema-toxylin and eosin. Magnification: 10 × 20, H604 Trinocular Unico, USA

Source: taken by E.M. Lenchenko, V.V. Ponomarev.

Pathological processes with prevalence of signs developing according to the type of reaction of hypersensitivity of the delayed type were accompanied by congestive hyperemia of vessels, massive disintegration of lymphocytes, erythrocyte diapadesis, disseminated thrombosis, toxic dystrophy of cardiomyocytes, alveolocytes, hepatocytes, nephrocytes. Infiltration of intestinal mucosa by pseudoeosinophils, polymorphonuclear leukocytes, macrophages was accompanied by perivascular edemas, apoptosis-inducing reactions of Harder glands, esophageal and ileocecal lymphoid follicles. Exudative-infiltrative processes, proliferation of sensitized lymphocytes, macrophage infiltration of thymus, Meckel's diverticulum, hyperplasia of spleen developed. The initiation, development and outcome of infectious process are mediated by stability of homeostasis of macroorganism of susceptible species and implementation of pathogenic potential of isolates producing adhesive antigens, bacteriocins, hemolysins, and cytotoxins.

Analysis of the results obtained and literature data indicate that a decrease in the population level of evolutionarily established microbiocenoses is accompanied by excessive growth of antibiotic-resistant microorganisms [8, 9, 15–17]. The phenotypes of isolates associated with septicemia of broiler chickens were resistant to ampicillin — 97.3%, tetracycline — 95.9%, spectinomycin — 95.9%, streptomycin — 93.2%, kanamycin — 89.0%, trimethoprim/sulfamethoxazole — 82.2%, chloramphenicol — 79.5%; oxacillin — 78.1% [18]. In gastrointestinal pathologies of poultry, more than 50.0% of isolates were resistant to azithromycin, lincomycin and enrofloxacin [19]. The causative agents of broiler coliform disease were resistant to drugs from six different classes, most often to tetracycline, ampicillin, gentamicin, and tobramycin [20]. In the development of new and rotation of existing chemotherapeutic and disinfectant agents, broad-spectrum antiadhesive composite drugs were recognized as promising [21–26]. In inducing an immune response, vaccines containing adhesive antigens isolated from bacterial cells and possessing high protective and preventive properties are more effective and less reactogenic [3–5, 27]. Along with the use of antibacterial, fungicidal drugs and specific prophylactic agents, drugs for correcting the immune status of the body are recommended [28–31].

## Conclusion

Decrease in natural resistance of mucosa-associated lymphoid tissue is associated with significant changes in systemic organization and consolidation of evolutionarily formed microbiocenoses due to increase in number and spectrum of pathogenic microorganisms. The initiation, development and outcome of syndrome of excessive growth of antibiotic-resistant microorganisms are mediated by hemodynamic disorders, pronounced vasodilation, activation of renin-angiotensin-aldosterone system, dystrophic and compensatory-adaptive processes. Excessive growth and translocation of antibiotic-resistant strains cause a variety of clinical manifestations and difficulties in differential diagnosis of infectious diseases, decrease in effectiveness of antibiotic therapy and anti-epizootic measures.

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## Динамика изменений бактериологических и морфометрических показателей при снижении колонизационной резистентности кишечника птиц

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**Аннотация.** При снижении компенсаторных механизмов естественной резистентности организма за счет изменений состава эволюционно сложившихся микробиоценозов возрастают риски развития синдрома избыточного роста антибиотикорезистентных микроорганизмов. Цель исследования — анализ динамики изменений бактериологических и морфофункциональных показателей при снижении колонизационной резистентности кишечника птиц. Для оценки количественного и видового состава микроорганизмов учитывали индекс колонизации бактерий, выделенных из содержимого слепых отростков кишечника клинически здоровых и больных птиц. Динамику изменений морфофункциональных показателей при диссеминации патогенных бактерий в ткани и органы исследовали с применением цитологических и гистохимических исследований. Количество микроорганизмов, выделенных из содержимого слепых отростков кишечника больных птиц, достоверно увеличивалось, индекс колонизации лактозоположительных бактерий — 0,247...0,283; лактозоотрицательных бактерий — 0,657...0,730. При избыточной бактериальной контаминации ileocecalного отдела кишечника и транслокации патогенов за пределы желудочно-кишечного тракта развивались признаки дистрофии, некроза и отторжения эпителиоцитов слизистой оболочки дыхательной и пищеварительной системы. Инициация, развитие и исход инфекционного процесса опосредованы стабильностью гомеостаза макроорганизма восприимчивых видов и реализацией патогенного потенциала изолятов, продуцирующих адгезивные антигены, бактериоцины, гемолизины, цитотоксины.

**Ключевые слова:** адгезивные антигены, бактериоцины, гемолизины, цитотоксины, диссеминация бактерий, дистрофия, некроз

**Вклад авторов:** концепция — Ленченко Е.М.; методология, валидация — Пономарев В.В.; работа с данными — Ленченко Е.М., Пономарев В.В.; ревизия и редактирование текста — Ленченко Е.М. Все авторы прочитали окончательную версию рукописи и согласны с ней.

**Заявление о конфликте интересов.** Авторы заявляют об отсутствии конфликта интересов.

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