организме. Роль минеральных элементов первой группы (кальция, фосфора, натрия, калия, железа, магния, марганца, цинка, кобальта и некоторых других) в жизнедеятельности животного организма хорошо изучена; необходимость их обязательного потребления животными в составе кормов сомнений не вызывает. Разработаны нормы содержания этих элементов в рационах и способы обогащения ими кормов. Что же касается элементов второй группы, к которой относятся фтор, кремний, титан, ванадий, мышьяк и некоторые другие элементы, то значение их для организма животных изучено недостаточно. Это не дает оснований отнести их к жизненно необходимым, поэтому их не учитывают при балансировании питательных веществ в рационе. В данной статье описаны результаты оценки токсикологических показателей кремнистой породы – опоки в лабораторных условиях.

UDC:619:616.98:579.852.11 ***DOI 10.52578/2305-9397-2024-1-1-184-192***
IRSTI:68.41.53

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STUDY OF THE MICROBIAL LANDSCAPE OF PERITONEAL EXUDATE IN ABDOMINAL INFLAMMATION

ANNOTATION

These studies reveal the features of the composition of microorganisms in peritoneal exudate in inflammatory processes, which can lead to more effective methods of diagnosis and treatment of such conditions. Inflammation of the abdominal cavity in rats of the experimental groups was caused by intraperitoneal administration of a suspension of coprological material (feces) at a dose of 0.5 cm^3.As a result of the conducted studies, it was found that on the 2nd and 5th days after infection of rats with coprological exudate into the abdominal cavity, inflammation of the abdominal organs developed, which manifested itself as an inflammatory process characterized by a disorder of blood and lymph circulation in blood and lymphatic vessels, in capillaries and venules, edema, hemorrhages in internal organs and vessels, necrosis tissues (on the 5th day after infection). In rats of the 3rd experimental group on the 3rd

day after infection the condition sharply worsened, there was oppression, refusal of food, the animals crammed into a corner of the cage and did not move. *Pr. mirabilis, Klebsiella spp., Listeria, Sarcina u E.coli.* were isolated from samples of biological material of rats. We studied the sensitivity to 7 antibacterial drugs of isolated microorganisms, among which were *Pr.mirabilis* and *Klebsiella spp.* isolated from the abdominal cavity during inflammation. As a result of the experiment it was found that the highest sensitivity in inflammation of the abdominal cavity in rats observed highly sensitive to fluoroquinolone antibiotics, cephalosporins, and resistance to aminoglycosides and tetracycline.

***Key words**: peritoneum; inflammation; antibiotics; nutrient medium; sensitivity to antibiotics.*

Introduction. The peritoneum is a serous membrane that covers the walls, many organs, is an extensive serous organ with epithelial and mesenchymal features and many functions [1]. Many diseases, such as inflammatory peritonitis and diseases of the abdominal cavity, can cause a violation of complex physiological functions. Despite the successes in the field of healthcare, peritonitis is still one of the most severe complications of inflammatory diseases of the abdominal cavity[2]. However, despite the large arsenal of antimicrobial drugs, there is no significant reduction in the mortality rate, which indicates that this problem is unresolved. For in-depth understanding of the course of the infectious process, taking into account the danger of its generalization, it is of great importance to study the translocation of microbial flora, which is one of the main mechanisms of pathogenesis in common forms of peritonitis [3]. Experimental models are used to test new ways of treating widespread purulent peritonitis. Microbiological control plays an important role in assessing the severity of the course of simulated widespread purulent peritonitis [4,5]. Bacterial infections continue to occupy one of the leading places in the infectious pathology of animals and humans [6]. In conditions of purulent inflammation of the peritoneum, an imbalance develops between different types of microorganisms and their distribution in different parts of the intestine. The microbial factor in RP is the main one and acts as a "trigger mechanism" for subsequent physiological disorders [7]. As a result of damage and/or infection of tissues in the human body, a complex and multicomponent sequence of reactions develops aimed at preventing further tissue destruction, isolating and destroying the pathogenic factor, activating reparative processes and restoring the original homeostasis [8]. There are 4 degrees (phases) of intestinal microbiological ecosystem disorders characteristic of enteral insufficiency syndrome in conditions of peritonitis [9,10]. In the first (initial) phase of the pathological process, there is a sharp decrease in the number of symbionts in their natural habitats.In the second phase, the species ratio of the microflora changes with an increase in the number of certain bacteria (*Escherichia, Klebsiella and Enterococcus*) [11]. In the third phase of the pathological process, the localization of autochthonous microflora changes — it moves to areas of the intestine where it has not previously been encountered.The process of so-called proximal microbial contamination or colonization is developing. In the fourth phase, the presented microbial associations, moved proximally through the digestive canal, show signs of pathogenicity [12]. The widespread use of antibiotic-resistant pathogens that cannot be treated indicates the urgency of the problem. In this study, we focus on the analysis of the microbial landscape of peritoneal exudate in the case of inflammatory processes in the abdominal cavity [13]. For the first time, the microbial landscape in rats infected intraperitoneally with a suspension of feces was studied. Modeling of scatological peritonitis in rats was carried out, and the body's immune response was studied. During a bacteriological study of biomaterial from rats with experimental peritonitis, pathogenic and conditionally pathogenic microorganisms were isolated. All experimental animals were clinically healthy. The sensitivity of isolated cultures of microorganisms to antibiotics of various groups was studied. The effectiveness of treating infectious diseases of bacterial etiology with antimicrobial drugs depends on the correct choice of the drug, taking into account the sensitivity of the pathogen to it, and the choice of the optimal dose. The preliminary diagnosis was established based on epidemiological, clinical and pathological data, and the final diagnosis was based on the results of bacteriological examination. Irrational use of antibiotics, often using maximum doses, increasing the course of treatment and frequency of use of drugs without taking into account sensitivity, as well as the pharmacokinetics of drugs, leads to the development of adverse reactions, the formation of drug sensitivity of pathogens and bacterial carriage. The purpose of the researchis experimental modeling of scatological inflammation of the abdominal stripe in rats, studying the body’s immune response.

The purpose of this work is to investigate the characteristics of the composition of microorganisms in peritoneal exudate during inflammatory processes, which can lead to more effective methods for diagnosing and treating such conditions.

Materials and methods of research. Studies on creation of experimental peritonitis on biological system in vivo on laboratory rats were carried out. In accordance with the aim and objectives of the program 12 rats - Spraque Dawley (SD) line sexually mature males and weighing 270±5 g were used in the experiments. The purpose of the work is experimental modeling of scatological inflammation of the abdominal stripe in rats, studying the body's immune response. For the experiment, 2 experimental and 1 control groups of animals were formed. There were 4 rats (male) in each group. 1st control group (4 heads) - uninfected animals that did not receive treatment; 2nd experimental group (4 heads) – rats infected with fecal suspension at a dose of 0.5 cm^3 intraperitoneally, killed 2 days after infection; 3rd experimental group (4) - rats infected with fecal suspension at a dose of 0.5 cm^3 intraperitoneally, killed 5 days after infection. The animals were kept in the vivarium on standard feeding and drinking regimen. Each experimental and control groups of animals were kept in a separate cage provided with individual drinker and feeding. During the experiment all rats received a complete balanced diet. Before the beginning of the experiment all rats of experimental and control groups were examined, weighed, and thermometered.

Acute inflammation of the abdominal stripe in rats of the experimental groups was caused by intraperitoneal administration of a suspension of scatological material (feces) in a dose of 0.5 cm^3 10% suspension was prepared from fresh scatological material taken from rats in sterile boxing conditions in physiological solution. Animals of all experimental groups were injected intraperitoneally with 0.5 cm^3 of a 10% suspension of scatological material. The animals of the experimental and control groups were observed for 5 days. When slaughtering, samples of biological material were taken from rats in the experimental and control groups for bacteriological examination. Abdominal exudate, abdominal flush and a 20 g piece of liver were taken into sterile containers for microbiological analysis. Cultures from samples of biological material from rats onto nutrient media were carried out immediately after collection.

When performing the work, bacteriological, serological, and biochemical research methods were used. When setting up the experiment, the physiological characteristics of the animal's body were taken into account [8]. After slaughter, a pathological examination of the rat corpses was carried out [9]. Biomaterial sampling was carried out in accordance with the methodological recommendations for sampling [10].

The rat corpses were opened in sterile boxing conditions and collected pieces of liver, abdominal exudate, feces, etc. were placed in sterile Petri dishes. The liver at the puncture site was cauterized with a Pasteur pipette, tissue was collected into a Pasteur pipette and inoculated into test tubes with a nutrient medium. In sterile box conditions, a bacteriological study of biomaterial samples from slaughtered rats was carried out by seeding on liquid and solid nutrient media. The material was sown on solid and liquid nutrient media. Crops were done on MPB, MPA (Russia) and on special nutrient media (for cultivating intestinal bacteria on Endo medium, on Chromagard for cultivating coccal microflora, etc.). After 20 hours of cultivation at 37°C in a thermostat, the crops were examined visually, suspicious colonies were selected and smears were made. Bacteriological examination of biomaterial from rats was carried out in accordance with the "Guidelines for Bacteriological Diagnostics". Identification and taxonomic distribution of the isolated crops were carried out in accordance with Bergey's identification guide [11]. The cultural and morphological properties of microorganisms were studied by inoculation on MPS, MPA (Russia) and differential diagnostic media. Microscopy was carried out on smears prepared from daily agar cultures, Gram-stained. The biochemical properties of microorganisms were studied by sowing isolated cultures on Hiss media with carbohydrates.The mobility of microbes was determined by growth on semi-solid agar [12].

To determine sensitivity to antibiotics, a daily broth culture of microorganisms that was not contaminated with foreign microflora was used. We used standard paper disks with antibiotics (2 mcg, 5×50 BioVitrum, Russia). The sensitivity of microorganism cultures isolated from animals was studied using the disk diffusion method in accordance with generally accepted methodological recommendations [13, 14]. 25 cm^3 of MPA was sterilely poured into sterile Petri dishes with a diameter of 100 mm, culturing with test microorganisms (CFU 10^6/mL). Before sowing, Petri dishes with agar were well dried in a thermostat for 48 hours. A bacterial suspension (a daily broth

culture) in an amount of 0.1 cm3 was applied to the surface of the agar and evenly distributed with a spatula, after which discs soaked in various antibiotics were applied with sterile tweezers. In each cup, the effect of 7 antibiotics was tested. After application of the discs, the Petri dishes were incubated at a temperature of 37°C for 18-20 hours upside down. The results were assessed by the presence of zones of inhibited growth of microorganisms around the discs [15, 16, 17, 21]. The absence of growth of the microorganism at a distance of more than 15 mm from the disk with the antibiotic indicated the sensitivity of the culture to this antibiotic. If the test microorganism developed in close proximity to the disk impregnated with the antibiotic, then this microorganism was assessed as resistant to the action of the antibiotic [18, 19]. The diameter of growth inhibition zones, taking into account the diameter of the disc itself, was measured with an accuracy of 1 mm. For quality control, test strains *Pr. mirabilis* and *Klebsiella spp.* were used [20, 22].

Results and discussion. One of the main reasons for the development of inflammatory process in the abdominal cavity is gastrointestinal (GI) autoinfection, in which the type of microorganisms and their virulence are a determining factor in the peculiarities of the development, clinical manifestations and outcome of the disease. It is known that the population of GI microbes includes about 40,000 species, and only 30-40 of them account for almost 98-99% of the intestinal microbiota. When an inflammatory process occurs in the abdominal cavity, the intestinal barrier function is impaired. Reduced intestinal barrier function leads to microbial (bacterial) translocation, in which some viable microorganisms and their toxins are able to overcome the damaged intestinal wall. A small number of microorganisms are able to overcome the intestinal mucosa outside the pathology, but when they get into the general bloodstream they are destroyed by liver kupffer cells. The first clinical symptoms of the disease in animals of 2nd and 3rd experimental groups appeared on the second day after infection. The animals were lethargic, refused food, did not move, were crowded, and there was a slight swelling in the abdominal area. Anaemia of visible mucous membranes was observed in rats of both groups during clinical examination. In rats of the 3rd experimental group on the 3rd day after infection the condition sharply worsened, there was oppression, refusal of food, the animals crammed into a corner of the cage and did not move. Exudate from the abdominal cavity, flush from the abdominal cavity and a piece of liver weighing 20 g were taken from the killed rats for bacteriological examination.

Sowings from the exudate from the abdominal cavity, abdominal flush from the experimental groups were made on MPB, MPA. The cultures were cultured for 20 hours in the thermostat at 37°C. After 20 hours of cultivation, the cultures were visually inspected for suspicious colonies. In the cultures from all samples of biomaterial (exudate from abdominal cavity, flush from abdominal cavity, liver) from 4 rats of the 3rd experimental group, abundant growth of *Proteus mirabilis* was observed. On MPB, a uniform opacity without a ring or mucous precipitate was observed. *Proteus mirabilis* on MPA grew as small round, matt, opaque, opaque, convex colonies with smooth edges, white or yellowish in H-form. Gram stained smears showed Gram-negative bacilli arranged in pairs or in chains, not forming a capsule (Fig. 1). The diagnosis of Proteus infection was established on the basis of the culture and morphological characteristics of the pathogen.

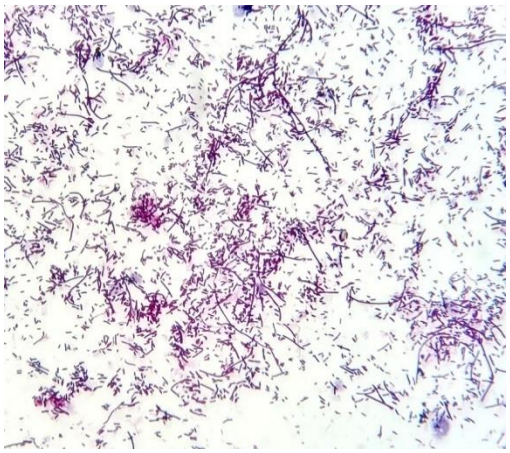

Figure 1 – *Proteus mirabilis* in a Gram-colored smear

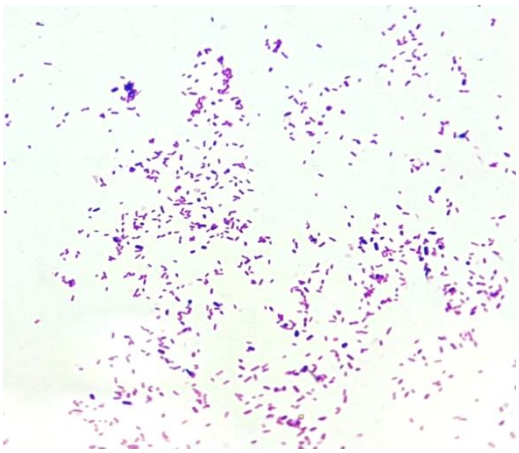

Figure 2– *Klebsiella spp.* in a Gram-colored smear

The growth of *Klebsiella spp.* was observed in cultures from the abdominal cavity flush of rats of the 3rd experimental group. On MPA *Klebsiella spp*. grew in the form of convex large colonies with even edges of yellow-green colour. On MPB, uniform opacity was observed. Gram stained smears showed small Gram-negative bacilli arranged singly, in pairs, in chains (Fig. 2). In smears prepared from daily agar cultures and Gram stained, large Gram-negative bacilli were observed, located singly in the smear, typical for *E.coli.* On MPA, *E.coli* grew in the form of large round slimy colonies of yellowish colour. And also, 2nd experimental group was observed growth of *Listeria* colonies on dense nutrient medium small, with raised edges, with pointed or raised centre, edges of colonies smooth, surface rough with shiny white or bluish tint (S-form) (Fig. 3).

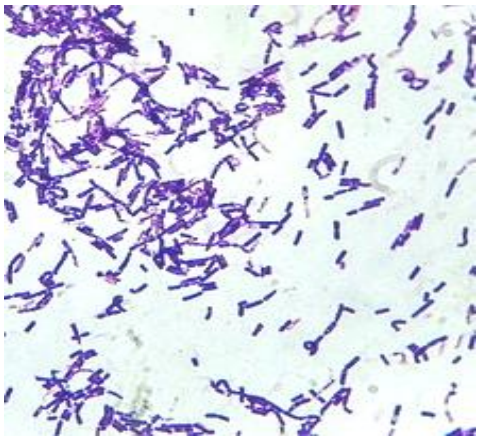

Figure 3– *Listeria* in a Gram-colored smear

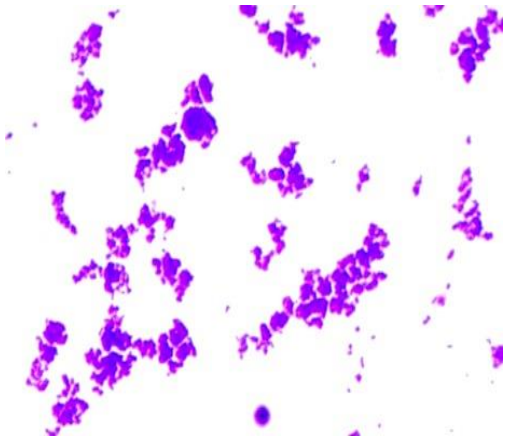

Figure 4– *Sarcina* in a Gram-colored smear

Sarcina colony growth was observed in 3rd experimental groups. On dense media they form round smooth colourless or yellow, orange, red colonies, which is due to the presence of carotene pigment in the cells. Gram-positive bacteria were observed in Gram stained smears (Fig. 4).

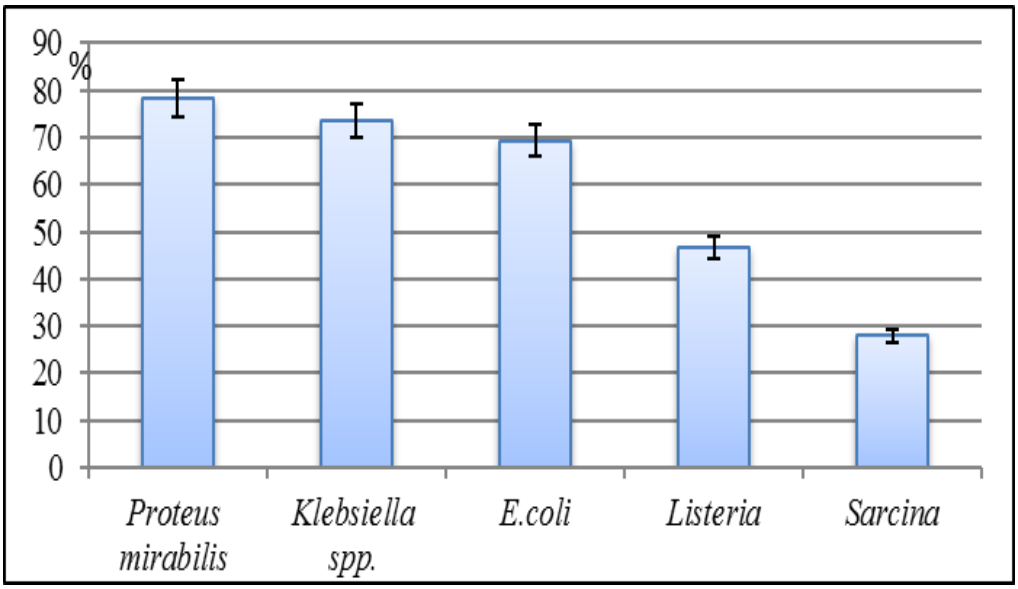

Figure 5 – Frequency of microbial isolation from the abdomen during inflammation

The frequency of microorganisms isolated was as follows: *Pr. mirabilis* was found in 78.3%, *Klebsiella spp.* - 73.5%, *E.coli* - in 69.4%, *Listeria* - in 46.7%, *Sarcina* - in 27.9% (Fig. 5).

Antibiotic sensitivity. We studied the sensitivity to 7 antibacterial drugs of isolated microorganisms, among which were *Pr.mirabilis* and *Klebsiella spp.* isolated from the abdominal cavity during inflammation. The results of the studies are summarised in the table.

Table 1 – Antibiotic sensitivity of microbial strains isolated from the abdominal cavity during inflammation

№	Antibiotic	Contents on disk, mcg	Growth retardation zone, mm	Sensitivity
1	Norfloxacin NX[10]	10	30	S
2	Ofloxacin OF[5]	5	32	S
3	Gentamicin GC[30]	30	23	R
4	Tetracycline TE[30]	30	8	MS
5	Ceftriaxone CFX[5]	5	27	R
6	Ciprofloxacin CFL[5]	5	15	MS
7	Lomefloxacin LOM[10]	10	34	S
Note: S - sensitive; MS - moderately sensitive; R - resistant				

High sensitivity of isolated microorganisms to lomefloxacin and norfloxacin was noted (Table 1). The table shows that all strains of *Pr. mirabilis* isolated from the abdominal cavity during inflammation showed sensitivity to fluoroquinolone antibiotics (norfloxacin (10 mcg/disc), lomefloxacin (10 mcg/disc), ofloxacin (5 mcg/disc), moderately sensitive to tetracycline (30 mcg/disc), ciprofloxacin (5 mcg/disc), gentamicin (30 mcg/disc). And cultures of *Klebsiella spp.* showed resistance to ceftriaxone (5 mcg/disc), lomefloxacin (10 mcg/disc), moderately sensitive to ciprofloxacin (5 mcg/disc), gentamicin (30 mcg/disc).

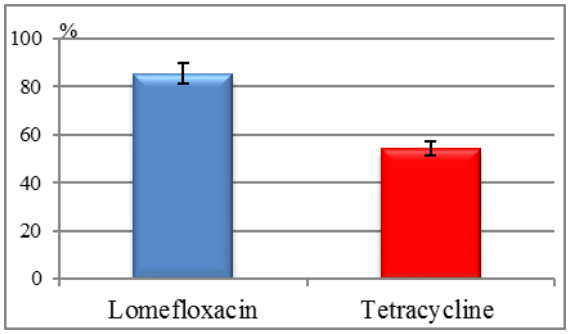

Figure 6 – Comparison of antibiotic sensitivity of *Pr. mirabilis*

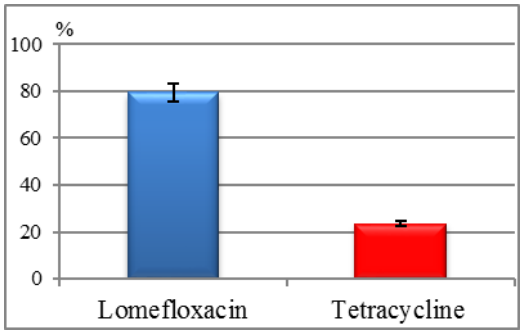

Figure 7 – Comparison of antibiotic sensitivity of *Klebsiella spp.*

Pr. mirabilis and *Klebsiella spp.* were highly sensitive to fluoroquinolones, a group of drugs with pronounced antimicrobial activity, widely used in medicine as broad-spectrum antibiotics (Fig. 5, 6). They are indeed close to antibiotics in terms of the broad spectrum of antimicrobial action, activity and indications for use, but differ from them in chemical structure and origin. Our study demonstrates high sensitivity of lomefloxacin to microorganisms isolated from the abdominal cavity during inflammation. *Klebsiella spp.* isolated from the abdominal cavity during inflammation, not only in 100% of cases is a producer of BLRS (plasmid beta-lactamases of extended spectrum), but also has extremely high associated resistance to antibacterial drugs of other groups - aminoglycosides (86.7%), fluoroquinolones (82.6%). Isolated from biomaterial from rats, showed moderate sensitivity to aminoglycosides (gentamicin).

Thus, the results of laboratory studies showed high efficacy of antibiotics lomefloxacin and norfloxacin against *Pr.mirabilis* and *Klebsiella spp.* bacteria, the causative agent of bacterial inflammation of the abdomen, indicating that they can be used against this disease.

Conclusion. As a result of bacteriological study, the pathogens of purulent-septic infections *Pr. mirabilis*, *Klebsiella spp., Listeria, Sarcina* and *E.coli* were isolated from all samples of biological material from rats of the experimental group. Pathological anatomical examination in rats revealed pathological and degenerative changes in the abdominal cavity characterised by accumulation of serous-purulent exudate, degenerative tissue degeneration, massive haemorrhages and foci of necrosis. As a result of the experiment it was found that the highest sensitivity in inflammation of the abdominal cavity

in rats observed highly sensitive to fluoroquinolone antibiotics, cephalosporins, and resistance to aminoglycosides and tetracycline. The results of laboratory studies showed high efficacy of antibiotics lomefloxacin and norfloxacin against *Pr. mirabilis* and *Klebsiella spp.* bacteria, the causative agent of bacterial inflammation of the abdomen, indicating that they can be used against this disease.

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ТҮЙІН

Бұл зерттеуде қабыну үдерісі кезіндегі перитонеалді экссудаттағы микроорганизмдер ерекшеліктері анықталып, қабыну кезінде диагностикалау мен емдеудің тиімді әдістері зерттелді. Тәжірибелі топтардың егеуқұйрықтарындағы іш қуысының қабынуы копрологиялық материалдың суспензиясын 0,5 см^3 дозада құрсақішілік енгізу арқылы туындады. Зерттеулер нәтижесінде егеуқұйрықтарды копрологиялық экссудатпен жұқтырғаннан кейін 2-ші және 5-ші күндері іш қуысында қабыну үрдісімен көрінетін эксперименттік қабынудың дамығаны анықталды, қан мен лимфа тамырларындағы, капиллярлар мен венулалардағы қан және лимфа айналымының бұзылуымен, ісінумен, ішкі ағзалар мен тамырларда қан кетулермен, ұлпалардың некрозымен (инфекциядан кейінгі 5-ші күні) сипатталады. 3-тәжірибе тобының егеуқұйрықтарында инфекциядан кейін 3-ші күні жағдайы күрт нашарлап, депрессия пайда болды, тамақтан бас тартты, жануарлар тордың бұрышына тығылып, қозғалысы баяулап қалды. Егеуқұйрықтардан алынған биологиялық материал үлгілерінен *Pr. mirabilis, Klebsiella spp., Listeria, Sarcina* және *E.coli* бөлініп алынды. Құрсақ қуысының қабынуы кезінде бөлініп алынған микроорганизмдердің, соның ішінде *Pr.mirabilis* және *Klebsiella spp.* 7 антибиотикалық препараттарға сезімталдығы зерттелді. Тәжірибе нәтижесінде егеуқұйрықтарда құрсақ қуысының қабынуына ең жоғары сезімталдық фторхинолондық антибиотиктерге, цефалоспориндерге, сондай-ақ аминогликозидтерге және тетрациклинге төзімділікпен жоғары сезімталдықпен байқалғаны анықталды.

РЕЗЮМЕ

Проведенные исследования раскрывают особенности состава микроорганизмов в перитонеальном экссудате при воспалительных процессах, что может привести к разработке более эффективных методов диагностики и лечения подобных состояний. Воспаление брюшной полости у крыс опытных групп вызывали внутрибрюшинным введением суспензии копрологического материала в дозе 0,5 см^3. В результате проведенных исследований установлено, что на 2 и 5 сутки после заражения крыс копрологическим экссудатом в брюшной полости развивалось воспаление органов брюшной полости, которое проявлялось в виде воспалительного процесса, характеризующегося нарушением крово- и лимфообращения в кровеносных и лимфатических сосудах, в капиллярах и венулах, отеком, кровоизлияниями во внутренних органах и сосудах, некрозом тканей (на 5 сутки после заражения). У крыс 3-й

опытной группы на 3-е сутки после заражения состояние резко ухудшилось, появилось угнетение, отказ от еды, животные забились в угол клетки и не двигались. Из проб биологического материала крыс были выделены *Pr. mirabilis, Klebsiella spp., Listeria, Sarcina* и *E.coli.* Изучена чувствительность выделенных микроорганизмов к 7 антибактериальным препаратам, среди которых *Pr.mirabilis* и *Klebsiella spp.* выделяют из брюшной полости при воспалении.В результате эксперимента было установлено, что наибольшую чувствительность при воспалении брюшной полости у крыс наблюдали при высокой чувствительности к фторхинолоновым антибиотикам, цефалоспоринам, а также устойчивости к аминогликозидам и тетрациклину.

УДК 619:616.98:578.835.2:078:616:33 ***DOI 10.52578/2305-9397-2024-1-1-192-201***
МРНТИ 62.41.09, 62.41.31

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