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PERINATAL LISTERIOSIS: THE MOUSE MODEL

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The Gram-positive bacterium *Listeria monocytogenes* is typical sapronontic pathogen. *L. monocytogenes* causes listeriosis, a severe disease with multiple manifestations including stillbirths and meningitis of newborns, in humans and a wide range of domestic and wild animals. The invasion factor of the internalin family InlB is involved in crossing the maternal-fetal barrier (Disson et al., 2008). Previously, we compared human and wild animal *L. monocytogenes* strains and described several naturally occurring InlB variants. We demonstrated that InlB variants differed in the ability to support intragastric infection in mice (Sobyanin et al., 2017). The aim of this work was to compare effects of InlB variants on perinatal infection. The mouse model was used. The InlB variants differing in 10 amino acid substitutions were expressed under the same promoter in the *L. monocytogenes* strain EGDeΔinlB. Work with animals was performed with approval of local bioethical committee. Mice were intragastrically infected on the 14th day of pregnancy, euthanized 1 and 3 dpi, bacterial loads were determined by plating. One of two InlB variants provided infection of both placentas and fetuses while another did not. Bacteria carrying InlB variant 14 but not the variant 9 were revealed in placentas 24 and 72 hpi. 65% of placentas and only 20% of fetuses were infected. Fetus infections was correlated with placenta infection. Infection was unequal for different fetuses in the same animal with bacterial loads ranges from individual bacteria to 10^5 CFU per fetus. Obtained results suggested that some but not all InlB variants might promote perinatal infection upon intragastric infection and that the infection of each placenta happens individually.

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PHENOTYPES AND GENOTYPES OF CLASSICAL AND HYPERVIRULENT *KLEBSIELLA PNEUMONIAE* CLINICAL STRAINS ISOLATED IN MOSCOW IN 2013–2018

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Klebsiella pneumoniae is causative agent of community-acquired and healthcare-associated infections including pneumonia, bloodstream infection, surgical site infections, liver abscess and meningitis. Multidrug resistant (MDR) *Klebsiella* belonged to classical branch of *K. pneumoniae* (cKp) have been included recently into the ESKAPE group of pathogens. On the other hand, in the last two decades hypervirulent *Klebsiella* phylogenetic branch (hvKp) emerged and spread around the world. In this study, we aimed to investigate phenotypes and genotypes of virulence and antibacterial resistance for 500 *K. pneumoniae* clinical strains collected in 2013–2018 from the patients of several Moscow hospitals.

Virulence factors and antibiotic resistance profiles between classical and hypervirulent *K. pneumoniae* isolates were compared. It was shown that hvKp strains were attributed to international sequence types ST23, ST86, ST65, and to novel clones ST2174 and ST2280, while strains of cKp — to international clones ST218, ST395, ST11, ST39, ST48, ST147, ST833, ST20, ST13, and ST3346. All hvKp strains had hypermucoviscosity phenotype, capsule types of K1, K2 and K57; carried 5–7 pathogenic genes (regulator of mucoid phenotype gene *rmpA*, aerobactin gene *aer*, iron uptake system gene *kfu*, allantoin metabolism gene *allR*, lipopolysaccharide synthesis genes *uge2* and *wabG*, and fimbrial gene *fimH*). On the contrary, cKP strains had no hypermucoviscosity phenotype, identified capsule types were K57, K62, K47, K14, K27, K28, K60, and K420. Such strains carried four or less pathogenic genes (they did not have *rmpA*, *aer*, and *allR* genes).

Major cKP strains in this study expressed the MDR phenotype, resistance to three or more classes of antibacterials; while more than 50% of them were resistant to six or more classes (beta-lactams, aminoglycosides, fluoroquinolones, sulfonamides, nitrofurans, phenicols). Molecular mechanisms of MDR include beta-lactamase genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA}, and bla_{NDM}), class 1 integrons carrying gene cassettes (*dfr, aac, aad,, aph,* etc.), and efflux pumps (*oqxAB, mph, cml,* etc.). Among hvKp strains two groups were described: (1) most of them were mainly susceptible to antibacterials carrying few resistance genes, (2) some strains accepted MDR plasmids carrying resistance genes, and expressed MDR phenotype.

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RELATIONSHIP BETWEEN MICROORGANISMS IN THE VAGINAL BIOTOPE OF SUBFERTIL WOMEN

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The aim of investigation was to evaluate the features of microecology of the lower genital tract of women with infertility.

A retrospective analysis of microbiological data of the vaginal discharge of 345 subfertil women was carried out. To assess the share of different types of microorganisms in the structure of the microbiota the coefficient of species constancy was used. To quantify the interaction between members of the microbiocenosis, the Jacquard similarity coefficient was used.

The nature of the relationship between the main members of the microbial community in the vaginal biotope of women with infertility should be considered antagonistic. The phenomenon of mutualism was characteristic only between *Lactobacillus* spp. and *Peptostreptococcus* spp. Typical *E. coli* have a significant ecological community with these bacteria, the relationships of their can be characterized as synergistic. Similar ecological synergism was revealed for *S. epidermidis* and lactobacilli. It was shown that in subfertil women *E. coli* acquires the functions of stabilizing strain and its activity often associated with both a change in the species composition of lactobacilli and their functional characteristics. In a similar situation despite the prevalence of microbial antagonism in the vaginal microbiota, *Lactobacillus* spp. "admit" the existence of *E. coli*, *Enterobacter* spp., *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. agalactiae*, *Enterococcus* spp. and *C. albicans.* Under such conditions, the negative influence of commensal microorganisms on lactobacilli is enhanced and its have manifestation by a marked decrease in their numbers and functional activity, as well as a decrease in the sensitivity of the associates to the biocidal factors of lactobacilli when coexisting.

Thus, the microecological approach to assessing the state of the microbiota provides the necessary information on the relationship between individual microorganisms in its composition and can be a valuable tool in deciphering the mechanisms for reducing fertility associated with nonspecific infections.

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IMPROVEMENT OF BACTERIAL BIOFILM'S INVESTIGATION

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Existing approaches to the investigation of biofilms haven't a unified methodology. Researchers use different solvents, allow deviations from the original technique. Each of them distorts the results and doesn't allow them to be compared.

The aim of investigation was to evaluate the possibility of using alcohol and acetic acid for dissolution of crystal violet, as well as additional dye Lugol's solution for the coloring biofilms.

The studies were carried out on *S. aureus*, *S. epidermidis*, *E. coli*. To form biofilms, the strains were cultured in flat-bottomed plates for 48 h. Biofilms were stained with a 0.1% solution of crystal violet (CV). In part of the studies after the coloration of biofilm with CV, Lugol's solution (LS) was used for 2 min. Extraction of dyes was performed with 70 and 95% alcohol and 33% acetic acid. Results were taken into account by measuring the optical density of solutions at a wavelength of 590 nm. Statistical analysis of the data was carried out using the paired version of the Student t-test. The threshold value was taken as $p < 0.05$. The data are given as the arithmetic mean (M) and its error (m).

It was shown that the use of 70% alcohol qualitatively elutes CV from biofilms than 95% one. This may be due to the fact that 95% alcohol narrows the pores of the cell wall and the dye is less efficiently released into the solution. The acetic acid more efficiently elutes the CV from biofilms formed by grampositive microorganisms. The use of LS after staining by CV showed the best results, but only if the extraction was carried out with a solution of acetic acid prepared with alcohol. This approach seems most optimal. LS fixes the dye in the cell and dissolution with a mixture of alcohol and acetic acid makes it more possible.

It was shown that extraction of CV is best performed with 70% alcohol. When fixing CV by LS, it is more appropriate to use a solution of acetic acid in alcohol.

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EFFECT OF METAL OXIDE NANOPARTICLES ON THE EXCHANGE OF GENETIC MATERIAL BETWEEN BACTERIA

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The development of resistance to antibiotics in bacteria is one of the most serious threats to global public health. The spread of resistance genes to antibiotics is caused by the increase and misuse of antibiotics in medicine and in animal feed. Nanomaterialscan increase the efficiency of horizontal transmission of multiresistance genes localized in plasmids between bacteria. Some studies have indicated that nanomaterials can cause damage to bacterial membranes, possibly by forming reactive oxygen species and can deliver DNA or RNA molecules to animal or plant cells. It was shown earlier the increase in efficiency of horizontal transmission of multidrug-resistant genes localized in plasmids by alumina nanoparticles (NPs) [1]. In this study, we observed the effect of different metal oxide NPs on the horizontal transfer of antibiotic-resistance genes between different *Escherichia coli* strains.

For the analysis of plasmid horizontal junctions, NPs of metal oxides Ta_2O_5, HfO_2, Fe_3O_4, ZrO_2, TiO_2, Al_2O_3 were used at a concentration of 5 mmol/L. The studies were conducted between *E. coli* strains containing different plasmids with different resistant cassettes. The transconjugants growth after incubation with metal oxides NPs was fixed on medium with appropriate antibiotics. The effect of metal oxide NPs was compared with control samples without addition of NPs.

In this study, we showed that the addition of NPs metal oxides can enhance the efficiency of horizontal gene transfer between different bacteria.

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SONOCHEMICAL NANOSTRUCTURING OF ANTIBIOTICS IS A NEW APPROACH TO INCREASING THEIR EFFECTIVENESS AGAINST RESISTANT STRAINS

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One of the most urgent problems of modern medicine is bacterial resistance to antibiotics. Development of new treatment approaches is a laborious and expensive process. One of the strategies for developing new antimicrobial agents is a modification of existing antibiotics. Sonochemical nanostructuring of antibiotics can become a cheap alternative to modern complex methods. In this regard, the aim of this research was to analyse the antimicrobial activity of sonochemically-modified tetracycline against sensitive and resistant strains.

Escherichia coli Nova Blue TcR (with antibiotic resistance) and *E. coli* 292–116 (without drug resistance) were used in this study. Tetracycline, a broad-spectrum antibiotic, was modified using industrial sonicator UIP1000hdT (Hielscher, Germany). The effectiveness of antibacterial properties was estimated using the disc-diffusion method and spectrophotometry analysis of liquid cultures. The results were confirmed by flow cytometry after staining with propidium iodide and Syto-9 dyes. The antimicrobial action of the modified antibiotic solution during long-term storage has also been studied.

The ultrasound processing time determines the change in antimicrobial properties against both sensitive and resistant cells. As a result of sonochemical treatment, the effectiveness of antibacterial properties increases up to 25% against the resistant strain and up to 100% against the sensitive strain. The long-term storage at +4°C does not reduce the antimicrobial properties.

The obtained data shows that sonochemical modification of antibiotics can be a new promising and cheap approach to the development of new drugs effective for antibiotic therapy against drug resistance strains.