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Keywords: Sophora Flower–buds, extractant, biologically active substances

Testing of antimicrobial activity of preservatives for dental gels development 17-23

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Introduction. Given the widespread prevalence of oral diseases, especially pathologies of periodontal tissues and mucous membranes, among the population of different ages and the needs of the pharmaceutical market in topical drugs for their treatment, the development of new domestic dental drugs is relevant. The most common topical dosage form in dental practice is gels, which are characterized by good distribution on the tissues of the oral cavity, prolonging effect and high bioavailability. At the Industrial Technology of Drugs Department of National University of Pharmacy, two dental gels are being developed under the name "Cholident" and "Lysostom". Previous studies showed that freshly prepared gels satisfied the requirements of SPhU for microbiological purity. However, contamination of the samples by test-microorganisms caused their intensive growth, which proved necessity for introduction of antimicrobial preservatives into the gels. These adjuvants prolong the shelf life of pharmaceutical products, increase their resistance to spoilage after opening the package, especially in the case of using multi-dose containers, and, accordingly, prevent infection of the patient. The **aim** of this work was selection of effective antimicrobial preservatives in rational concentrations for the composition of dental gels "Cholident" and "Lysostom". **Materials & methods.** The following ingredients were selected as preservatives in the gels are being developed: benzoic acid, sodium benzoate, sorbic acid, potassium sorbate, methyl parahydroxybenzoate (nipagin), propyl parahydroxybenzoate (nipazol). The concentration of preservatives for all gel samples was 0.1 % and 0.2 %. They have much lower toxicity than others, are harmless to humans even in large quantities and are permitted for the preservation of food and pharmaceutical products. The selection of preservative for the composition of dental gels "Cholident" and "Lysostom" was based on the study of its antimicrobial activity. Preservatives effectiveness tests were carried out according to the method of SPhU 2.3, Ch. 5.1.3. According to the requirements of SPhU, the sterility test of culture medium and solvent: growth properties of culture medium (soy-casein culture medium – for bacterial cells growth and Saburo-dextrose medium without antibiotics – for fungal cells) and suitability test of methods for determining the total number of cells were performed. **Results & discussion.** Tests have shown that for dental gel "Lysostom" with preservatives nipagin, sodium benzoate, potassium sorbate, it could be noted that the obtained data satisfy the requirements of SPhU for oral drugs. Among the preservatives listed above, sodium benzoate at a concentration of 0.2 % had the highest antimicrobial activity. The results of antimicrobial preservatives efficacy determination in the composition of the dental gel "Cholident" proved SPhU eligibility for all samples. The combination of nipagin : nipazol had the highest antimicrobial activity against all test-microorganisms, and, taking into account the requirements of economy and safety, their use at a concentration of 0.1 % will be sufficient. **Conclusion.** Thus, tests of the antimicrobial preservatives effectiveness in the experimental samples of dental gels "Lysostom" and "Cholident" proved that all the studied preservatives, namely sodium benzoate, potassium sorbate, nipagin and combination nipagin with nipazol, benzoic acid and sorbic acid, had high antimicrobial efficacy and met the requirements of SPhU for oral drugs. Taking into account a slightly higher antimicrobial activity, the sodium benzoate at a concentration 0.2 % was selected as the most acceptable preservative for the gel "Lysostom". The combination nipagin : nipazol (3:1) was chosen as the most promising preservative for the gel "Cholident", which provided at a concentration 0.1 % stronger than other preservatives antimicrobial action.

Key words: dental gel, preservative, microbiological studies, antimicrobial activity

Using the HACCP method in quality risk management in the production of oromucosal gel 24-29

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Introduction. With the adoption in the EU of the regulatory document of the European Medicines Agency (European Medicines Agency) EMA / INS / GMP / 79766/2011 “Quality Risk Management (ICH Q9)” separate guidance was issued in Ukraine in 2011 - Instruction ST-N MOH 42-4.2: 2011 “Medicines. Quality Risk Management (ICH Q9)”. Pharmaceutical industry and regulatory bodies professionals can assess and manage risk using recognized risk management tools and / or internal techniques (e.g., standard working methods). Application of general principles and approaches of the Guideline “Medicines. Quality Risk Management (ICH Q9)”at the stage of pharmaceutical development, using appropriate risk management tools both in general to the manufacture of medicines and to individual processes is an effective measure of quality assurance for the developed medicinal product. The technology of the combined dental gel was developed taking into account its properties as a dispersed system, as well as the properties of the active and auxiliary substances that are part of it. In order to manage quality risks effectively, it is necessary to have data about the sustainability of the process. Critical process parameters that need to be managed or monitored to ensure the required quality of the drug should be identified and specified. **Materials and methods of the research.** The subject of the study was the technology and technological scheme of the production of a combined gel for the treatment of infectious diseases of the mucous membrane of the mouth and gums. Hazard Analysis and Critical Control Points (HACCP) were used to evaluate and manage risks in the manufacture of the new drug. The HACCP tool, the “Decision tree”, was used to establish critical control points. **Results of the research and discussion.** An expert group of experts conducted an analysis and evaluation of the danger of individual stages of the technological process and determination of the criticality of the controlled parameters. The group consisted of qualified specialists from the pharmaceutical development department, thefactoryworkshop for the production of soft medicines, the quality control department. Analysis of the technological scheme of production of combined dental, showed that almost all stages of the dental gel manufacturing process are critical and marked in grey. Using the tool “Decision tree”, we identified the critical control points of the technological process of gel production, and set the eligibility criteria. Risk factors were evaluated on the basis of two indicators: the likelihood of a hazard factor and the degree of risk created by that factor. The likelihood of a risk factor was as follows: unlikely, quitelikely, probably, very likely. The degree of risk was assessed on a ten-point scale. During the processing of the gel technology, certain critical control points were monitored with the aim of developing preventive and corrective actions in case of their fall outside the eligibility criteria. in the process of gel production, risk (physical factor) at the stages of metronidazole suspension preparation, preparation of gel base and gel directly is “Quite likely”. “Unlikely” occurrence of a dangerous factor at such stages as the weighing of active and auxiliary substances, the preparation of solutions and during the input control of raw materials. The risk level of each factor, ranging from 2 to 7 points, was determined and corrective and preventive actions were developed for all critical control points to prevent risks to the quality of the medicinal product. **Conclusions.** Based on the data obtained in the development of dental gel technology and using scientific knowledge and methodology of risk assessment by the method of HACCP, an analysis of technological scheme of its production was carried out. Process risks have been identified, critical control points have been identified and their allowed limits. For each control parameter, the probability of occurrence and the degree of risk were determined, and measures were proposed to prevent or eliminate the effects of the risk.

Keywords: quality risks, HACCP method, technological process, control critical points, dental gel.

Justification of conditions of salicylic acid introduction into emulsion ointment base composition 30-34

Zuykina E.V., Polovko N. P.

Introduction. The revival of extemporaneous production requires the updating of prescriptions, the improvement of the technology of drugs, the study of stability in the process of storage in order to extend the shelf life. Semi-solid dosage forms, which take more than 25 % of medicines prepared in pharmacies about the stock, are currently prepared on a vaseline base. It has many disadvantages compared to the modern emulsion bases that are widely used in European practice. Therefore, the creation and implementation of modern emulsion bases for

semi-soled dosage forms is a promising direction for the development of pharmaceutical compouding. To obtain the drug of good quality, it is necessary to provide a rational way of introducing the active substances to the base, so it is necessary to justify the parameters of the ointment with salicylic acid preparation in the conditions of pharmacies. **Material & methods** The samples of the emulsion bases of the first and second kinds, into the composition of which 20 % of salicylic acid was introsuced by the different technology, were investigated. Determination of the degree of dispersion of the oil phase and salicylic acid in the base was carried out using a "NIKON ECLIPSE CI-S" triocular digital usb microscope with a built-in camera (the lens 40 X / 0.65 160 / 0.17; eyepiece WD 0,56) with 40x magnification. **Results & discussion** Microscopic imaging of the test samples showed that the average size of crystals in the base of the first kind was 5 times larger than in the base of the second kind. A more uniform distribution of the drug substance in the sample with the base of the second kind was established. It is shown that the more complete dissolution and uniform distribution of salicylic acid in the base are observed when dissolving the drug substance in the emulsion base of the seconf kind. **Conclusion.** Physico-chemical and microscopic studies substantiated the method of introducing salicylic acid into the emulsion base. The dependence of the degree of dispersion and distribution of salicylic acid on the type of selected emulsion base is shown. It was shown that API is advisable to dissolve in oil, since it promotes less dispersion and a more homogeneous distribution of salicylic acid. The technological scheme of preparation of 20 % salicylic ointment in the conditions of pharmacy is developed, which is layed in the basis of the technological instruction.
Key words: technology, semi-solid dosage forms, microscopic examination, emulsion base.

Modern pharmacotherapy of chronic hepatitis C in patients who failed to achieve sustained virologic response 35-38

Kireyev I.V., Zhabotynska N.V.

Introduction. According to WHO experts, about 150 million people suffer from chronic viral hepatitis C (CHC), and 350,000 die annually as a result of liver damage by the hepatitis C virus (HCV). Ukraine is one of the countries with medium prevalence of CHC – about 3% of citizens are infected. CHC has become a treatable disease with the use of antiviral drugs (> 95%). To date, for the pharmacotherapy of CHC, a combination of pegylated interferon (PEG-IFN) with ribavirin and direct-acting antivirals (DAAs) are used. Pharmacotherapy of CHC using a combination of PEG-IFN and ribavirin has a relatively high efficiency, but it depends on the genotype of the HCV. Therefore, the use of DAAs is a priority in pharmacotherapy of chronic hepatitis C. Patients who failed to achieve sustained virologic response (SVR) are given a second course of treatment (retreatment). The decision on this is based on the following main positions: the nature of the previous response, the type of previous therapy and the potential for a new type of treatment, the severity of liver damage, the genotype of the virus and the presence of other prognostic factors and tolerance to previous therapy. **Material & methods.** The article analyzes the recommendations of the American Society of Infectious Diseases (IDSA) and the American Association for the Study of Liver Diseases (AASLD), in collaboration with the US International Anti-Virus Society (IAS-USA), as well as the WHO recommendation for repeated pharmacotherapy of CHC in patients who failed to achieve SVR. **Results & discussion.** To date, for re-treatment of CHC in patients receiving treatment without achieving a SVR, the different re-treatment regimens are recommended depending on the genotype of the HCV. An important problem during pharmacotherapy of patients with CHC is resistance to antiviral therapy. The amino acid polymorphism of NS3, NS5A and NS5B viral proteins in different HCV genotypes and subtypes, as well as the same strains of genotypes and subtypes that reduce DAAs efficacy, is referred to as resistance-related variants (RRV). However, antiviral therapy fails only when RRV is combined with other factors and features of the patient's body, decreased sensitivity to antiviral therapy, or insufficient duration of therapy. As seen in the recommendations for recurrent CHC pharmacotherapy, possible resistance to the protease inhibitor NS5A and to the NS3 protease inhibitors was considered. **Conclusion. The** results obtained from published sources indicate that current strategies for recurrent pharmacotherapy of CHC patients in most cases of unsuccessful pre-treatment allow the achievement of SVR using DAAs during re-treatment, including those regimens that have efficacy in resistance-associated variants.
Key words: chronic viral hepatitis C, re-treatment, sustained virologic response.

Photodynamic inactivation of *P. aeruginosa* strains in order to obtain multistage vaccine 39-42

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Introduction. One of the main pathogens of purulent-inflammatory production, while in the interior remains *Pseudomonas aeruginosa*. The pathogen has a serogroup landscape, uses a polyresistant of its own and high impurities to disinfectants, asepsis and factors that exist in most. When antibiotics are detected, the effectiveness is insufficient, and trust in the hymen is created on the early and burn surfaces, on catheters, in the formation of a chronic course, which is in overuse of products, and in the absurd, pneumonia, implant differences and problems with prosthetics, which still have to continue. The problem with creating highly effective vaccines against *Pseudomonas aeruginosa* infection is the availability of vaccine antigens that have shown a protective response independent of serotype and under the type of pathogen, and have been found to be malignant. toxic and non-reactogenic. Look for new products aimed at creating the most modern drugs that are studied in different countries. In Ukraine, there are no diagnostic drugs for the identification of the pathogen and the determination of specific antibodies, and vaccines for the prevention and treatment of *Pseudomonas aeruginosa* infection Obtaining such drugs of domestic production is promising, relevant and socially and economically justified. **Materials and methods.** The basis of our proposed method of obtaining a multistage *Pseudomonas aeruginosa* vaccine is the method of photodynamic disinfection of bacterial cultures. An important feature of this method is its universal effectiveness of inactivation of bacteria and viruses, regardless of serotype, phagovar. For the experiment, 7 regional strains of *P. aeruginosa* isolated from different habitats stored in purulent-inflammatory sites were studied. As drugs for obtaining samples of the vaccine used a commercial drug - "Pseudomonas aeruginosa bacteriophage" (FDUP "NGO" Microgen ", Perm). As photosensitizers used 1% solution of vikasol (manufactured in Ukraine, "Darnitsa") and 0.1% solution of riboflavin (manufactured in Ukraine, "Darnitsa"). To use the photodynamic effect, we used a photo-polymer stand-alone lamp "Lux" with a powerful luminous flux of 1200 mW / cm2 (power - 90 mW / cm2. Ultraviolet (UV) radiation was used in the laminar box with a bactericidal lamp.. The titer of a mixture of our adapted phages was determined (by the Appelman method), which allowed to determine the dose of phage required for testing the phagolysis technique in experiments with various additives (riboflavin, vikasol). **Results and discussion.** At a phage titer of 1: 10^6, complete lysis of test-culture pseudomonads was recorded after 2-18 hours of incubation at a temperature of + 35^0 C. It should be noted that the growth of single colonies still occurs when sowing from a mixture of pseudomonads + adapted bacteriophages for 3-5 days. . Without irradiation, these substances in any concentrations did not affect the growth of *Pseudomonas aeruginosa* cultures, while at certain doses (vikasol - 3.5 μg / ml, riboflavin - 0.2 μg / ml) and irradiation parameters there was a decrease or lack of growth of bacterial cultures after sowing from experimental samples. The type and parameters of crop irradiation were determined separately. The irradiation regime was determined experimentally on pure cultures of *P. aeruginosa* strains taken in different concentrations (from 10^1 to 10^9) CFU / ml, at different time intervals (5,0-10, 0-15, 0-20, 0-30, 0 - 40, 0 min), with different concentrations of riboflavin and vikasol, with the addition of bacteriophage samples and without its addition). The optimal parameters for irradiation of the samples are 20 minutes when using UV rays and 30 minutes - when using daylight photopolymer lamp. Significant advantages of one method over another are not presented. To increase the photosensitizing effect, the combined use of vikasol and riboflavin was used. The most promising way to obtain a phagolytic pseudomonas vaccine that is decontaminated and does not contain toxic fractions is the use of specific or adapted to candidate cultures *P. aeruginosa* bacteriophages, riboflavin and vikasol, followed by irradiation with light or photopopulation. The method of obtaining immunogens developed on the model of *P. aeruginosa* can be successful for obtaining vaccines from other bacteria - pathogens of purulent-inflammatory diseases (staphylococcus, streptococcus, Escherichia coli, Proteus, etc.) and the design of polyvalent vaccines.

Keywords: Photodynamic inactivation, *Pseudomonas aeruginosa*, multistage vaccine

Development of a combined diphtheria vaccine based on diphtheria anatoxin with microbial adjuvant in the light of modern strategies of vaccines development 43-49

Yelyseyeva I.V., Babich Ye.M., Zhdamarova L.A., Bilozersky V.I., Kolpak S.A.

The review article is devoted to the consideration of approaches to the practical implementation of the main provisions of modern ideas about the role and mechanisms of innate immunity for the development of vaccines. Initiation of the body's immune defenses occurs through the recognition of microbial pathogens using typical molecular structures associated with pathogens (PAMPs), mediated by image recognition receptors (PRRs), which are expressed by cells of the innate immune system. The so-called Toll-like receptors (TLR) play a central role in initiating immune responses. Other PRRs such as membrane-bound lectin C receptors (CLR), cytosolic proteins such as the nucleotide-binding oligomerization domain - NOD-like receptors (NLR) and RIG-I-like are involved in the recognition of PAMP and the control of innate immunity. receptors (RLR), AIM-2-like receptors, and a family of enzymes that function as intracellular nucleic acid sensors, including OAS and cGAS proteins also involved in the recognition of PAMP and control of innate immunity. Innate control of adaptive immunity is now an established paradigm. PRR determines the origin of antigens recognized by receptors expressed on T cells and B cells, as well as determine the type of infection with which collides with the body, and teaches lymphocytes to induce an appropriate effector class of the immune response. A modern branch of vaccinology is a new paradigm for the development of broad-spectrum prophylactic drugs based on trained immunity (TIbV). These are vaccines that induce the learning or training of innate immune cells, the essence of which lies in their long-term metabolic and epigenetic changes, which lead to an enhanced cellular response to the second antigenic stimulus by the same or unrelated specific microbial stimulus. Because trained immunity is typically triggered by PRRs, TIbV must be formed from microbial structures containing the appropriate PRR ligands, namely, PAMPs. Unlike conventional vaccines, which aim to obtain only specific responses to vaccine-associated antigens, TIbV aims to stimulate a wider range of reactions. Broad protection can be achieved by enhancing the nonspecific effector response of innate immune cells to pathogens and using the dendritic cells activation state to enhance the adaptation of the T cell response to both specific and unrelated antigens. The concept of TIbV is in its infancy, but a number of modern anti-infective vaccines, immunomodulators and vaccine adjuvants can already be considered from the standpoint of the TIbV category. Induction can be achieved in various ways that enhance immunity, which can be involved by bacteria, fungi (β-glucan) or metabolic "trainers", as well as some cytokines. A new paradigm for drug development and therapeutic interventions for the prevention and treatment of infectious diseases is also the defeat of bacterial virulence as an alternative to antimicrobial therapy. One of the many antivirulence targets is adhesion. If it is possible to suppress adhesion, accordingly, it is possible to suppress the corresponding infection. This approach forms the basis of anti-adhesive strategies that have been invented to prevent various bacterial infections. Thus, the development of vaccines that prevent the initial stage of infection is in line with the anti-adhesive strategy. A combined diphtheria vaccine based on native purified diphtheria toxoid (NODA) with an adjuvant of microbial origin is developed at the SI “IMI NAMN” at the stage of preclinical trials. As an adjuvant in the candidate vaccine, a preparation of C.diphtheriae, var.gravis, tox + native surface antigens is used, which have not undergone modification or even denaturation with chemicals and therefore contain molecular structures as similar as possible to natural PAMPs and provide targeted antigen stimulation of cells of the innate immune system, obtained by physical means of disintegration of microbial cells (ultrasound or electromagnetic radiation of extremely high frequency). Studies have shown that the nativeness of antigenic candidate drugs for adjuvants is a key element of their effectiveness. Experimental samples of diphtheria bacterial surface antigens have shown themselves as adjuvant for diphtheria toxoid, capable of replacing neurotoxic aluminum hydroxide, and immunomodulator, which increase phagocytic activity in the first and repeated antigenic stimuli, and also promote the release of nasopharyngeal mucosa of rabbits vaccinated with experimental samples of the combined diphtheria vaccine and infected with a culture of C. diphtheriae. The formation of an immunologically strong mucosal barrier is considered to be an effective strategy to prevent infection at the point of contact between microbes and the host. However, modern standards of vaccine technology usually apply only to pathogens that have already crossed the mucosal barrier. In contrast to licensed vaccines, vaccination on mucosal surfaces, including the oral route of administration of the vaccine, can successfully stimulate the humoral and cellular immune response in both systemic and mucosal areas of the entrance gate of infection to establish a broader and longer-lasting protection. Experimental samples of the combined diphtheria vaccine with bacterial adjuvant were tested using the oral route of administration of the vaccine in combined vaccination regimens, and, as the results of experiments showed, the adjuvant and phagocytosis stimulating effect of the studied candidate vaccines was maintained. The obtained data demonstrated the prospect of widespread use of oral immunization as the most natural, physically and psychologically painless way to administer vaccines both for emergencies in the diphtheria outbreak and to maintain collective diphtheria immunity by booster immunization. However, oral delivery is a complex task that requires a special composition to overcome harsh gastrointestinal environments and avoid the induction of tolerance to achieve effective protection, which requires detailed justification of oral vaccines, including key biological and physicochemical aspects of next-generation oral vaccines. Another way to increase the safety of diphtheria vaccines during repeated vaccinations, which opens up prospects for specific immune protection of persons with allergic reactions, was the experimental application of the principles of specific immunotherapy in vaccinations of experimental animals. The obtained results indicate that the previous oral administration of antigenic drugs C. diphtheriae prevented the development of allergic skin reactions in experimental rabbits with subsequent subcutaneous administration of diphtheria vaccines. Studies on the development of a combined diphtheria vaccine with an adjuvant of bacterial origin convincingly showed the immunogenicity of the obtained experimental samples of the candidate vaccine and the possibility of combining the efficacy and safety of the drug at a certain dosage and degree of purification of antigenic drug. But for further research, it will be essential to expand knowledge about the sensitive pathways of the innate immune system and to determine the rules of interaction that determine the functions of these pathways in the context of diphtheria infection. Special attention should be paid to the study of the protective effect of the drug, the study of patterns of formation of antibacterial diphtheria immunity and the determination of optimal ratios of NODA and bacterial antigen in the vaccine.

Key words: trained innate immunity, adaptive immunity, development of vaccines, bacterial adjuvants, C.diphtheriae, mucosal vaccines, specific immunotherapy.

The stay of humoral immunity in bacterial dysbiosis and bacterial vaginosis 50-56

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Introduction. The state of dysbiosis and bacterial vaginosis (BV) is characterized by the formation of both systemic and local immune deficiency, which corresponds to the increase in the number of pathogenic microbiota. An important reason of bacterial vaginosis’ development is local immunodeficiency corresponding with decreasing of colonization resistance of vaginal fluid. This phenomenon develops due to disturbance of normal vaginal microbiocenosis, secretion of antimicrobial substances and provision of normal immune defense. Recognition of the role and mechanisms of local immunodeficiency’s development can be very important scientific achievement in the field of microbiology, immunology and pathology of human vaginal microflora. However, nowadays ratio of systemic and local immune reactions in bacterial vaginosis isn’t revealed completely. Thus, the aim of the investigation was to determine the stay of humoral immunity according to the content of immunoglobulins (Ig) in the blood and vaginal fluid in different degrees of bacterial dysbiosis and BV. **Material and methods.** Data from 298 women were divided into groups according to index of pathogenic microbiota condition (IPMC) and the pathogenic microbiota indicator (PMI): normocenosis (n=53), dysbiosis I (n=128) and II degree (n=117), among the last allocated 83 patients with PMI>1 lg gE/sample, where was drawn diagnosis “Bacterial Vaginosis”. The criterion of exclusion there was presence of pathogenic microorganisms in vaginal epithelium scrapings. These representatives were: *Trichomonas vaginalis, Neisseria gonorrhoeae, Chlamydia*

trachomatis та *Herpes Simplex Virus 1,2*. Presence of leucocytes more than 15-20 cells in the field of vision in vaginal smears indicated inflammatory reaction also was the criterion of exclusion. Molecular genetic studies of posterolateral wall of the vagina epithelium scrapings was performed by Real-time polymerase chain reaction. A content of facultative and obligate anaerobic bacteria, myco- and ureaplasma, yeast-like fungi was studied quantitatively. With the help of Enzyme-Linked Immunoassay (ELISA) contents of IgA, IgM, IgG, IgG_2, secretory IgA (sIgA) were determined in blood and vaginal fluid. Spectrophotometry was used for quantitative evaluation of circulating immune complexes (CIC) contents in the blood and immune complexes in vaginal fluid (IC_{VF}). For descriptive statistics of data there were used arithmetical mean (M) and average error (mistake). Paired independent data samplings were compared according to Mann-Whitney U-test (U). Significance of all differences accepted when $p<0,05$. For statistic and regressive analyses package of software "Statistica 10" (StatSoft, Inc., USA) was applied. **Results and discussion**. While development of bacterial dysbiosis and BV there was observed progressive increasing of CD22 lymphocytosis, contents of IgM, IgG i IgG_2. In our investigations quantity of CD22+ lymphocytes was constantly larger in manifested dysbiosis in comparison with normocenosis. Maximal content of CD22+ lymphocytes was noted in BV. Contents of IgA, sIgA and CIC had tendency to decreasing. In general, it's possible to conclude that blood CIC level decreases according to the progressing of dysbiosis. Hence, its more level in 2 subgroup of 2 group and in 1 subgroup of 3 could indicate reactive changes in immune system. Content of IC_{VF} in I degree dysbiosis in comparison with normocenosis was not change significally. Simultaneously, in I degree dysbiosis and in 1-st subgroup of II degree dysbiosis this inex was significally more. In BV content of IC_{VF} was twice less than in normocenosis. These phenomena were synchronous with blood CIC levels, and reflected sharp parallel decreasing of CIC formation both in the bloodstream and in vaginal fluid during BV. Local immunodeficiency with immunoglobulins' (especially, IgA and sIgA) and IC_{VF} levels decreasing progressed while development of bacterial dysbiosis and BV also. Therefore, the stay of systemic humoral immunity in BV didn't correlate always with such one in vaginal fluid. **Conclusion**. Systemic humoral immunity while development of BV was changed, but it wasn't reflected completely the stay of defences in vaginal fluid. In general, there was present dissonance of these two systems' reaction: activation of systemic level and suppression on local level.

Key words: bacterial vaginosis, humoral immunodeficiency, vaginal dysbiosis

Genetic monitoring of endemic measles virus circulation in European countries 57-70

Kalinichenko S.V., Melentyeva K.V., Toryanik I.I., Zvereva N.V., Antusheva T.I., Buriachenko S.V.

Introduction. The measles virus is still one of the main causes of morbidity and mortality in children and adults and is a threat of infectious outbreaks in many countries around the world. The World Health Organization (WHO), at the 2015 meeting in Europe, set out to eliminate measles infection. To control the elimination of this disease requires the accumulation of genotyping data of the detected measles virus to interrupt the situation of endemic spread. All six WHO regions have set a target for combating measles. In order to monitor and evaluate the degree of endemic circulation of measles virus (MV), the transmission chains of the epidemiologically relevant variants of MV identified in Central and Western Europe are analyzed. More systematic molecular monitoring and recording of MV transmission data between many countries can help to create a meaningful picture of the process of eliminating the problem of the occurrence and spread of measles infection. **Goal.** To study whether molecular surveillance meets the challenge of eliminating measles infection with the assurance of molecular data quality, continuity and intensity of molecular monitoring and analysis of transmission chains in different geographical regions. **Material** & **methods.** Published articles, molecular program for external WHO quality assessment, WHO EUR central infectious disease information system, and WHO measles surveillance database. **Results & discussion.** According to the WHO standardized nomenclature using the nucleotide (nt) sequences of the N and H variable genes, wild-type measles viruses are currently divided into 24 genotypes. The most variable is the 450-nt variable coding sequence of the C-terminal portion of the N protein (N-450 region) and is used to differentiate detected MV for observation. Antigenic differences between measles virus strains - representatives of different genotypes are minimal, all known genotypes of the virus belong to one serotype. Since the beginning of molecular surveillance in Europe in the early 1990s, only two genotypes of MV (C2 and D6) have been identified, which have been spread throughout the region and are therefore called indigenous European genotypes. Molecular observation has shown that, over the years, the endemic genotypes C2 (IR / Kempten.DEU / 23.00) and D6 (IR / Berlin.DEU / 47.00) have changed rapidly with the circulating D7 genotype (IR / Mainz.DEU / 06.00) to the beginning of 2003. The imported measles virus of genotypes B3, D4, D5, D6, D8, D9, H1 appeared in Germany from 2005 to 2009 - 2010. Most cases were related to the measles virus of genotype D4, and its several sub-variants. According to the monitoring data, genotypes D8, B3, H1, D9, D4 have been circulating in the world in recent years (from August 2017 to July 2018). Of the 179 measles deaths reported in European countries during 2009-2018, 114 (64%) occurred during 2017-2018, including 93 (82%) in four countries: Romania (46), Ukraine (20), Serbia (15) and Italy (12). EU countries report 17587 measles virus sequences to the WHO global measles surveillance database. The most common measles virus genotypes were D4 (21% overall, 66% in 2009-2012), D8 (45% overall, 76% in 2013-2016) and B3 (33% overall, 58% in 2017- 2018). **Conclusion**. Our research illustrates the long-term transmission of MV in Europe. Which probably happens because of the unvaccinated people in the various hard-to-reach groups that transmit the infection to the general population. This situation is, of course, inconsistent with the purpose of the WHO and UNESCO (WHO-UNICEF, 2002) measles elimination program already achieved in America and Australia. In order to address the global problem of measles infection worldwide, additional efforts are needed to identify deficiencies in immunization among the population. As the elimination of MV should be a problem for all EUR countries, similar research to ours should be expanded to obtain comprehensive information on the circulation of MV strains in different regions across Europe.

Keywords: Genetic monitoring, measles, circulation, Europe

The effect of *Bifidobacterim bifidum* cell-free supernatants, ascorbic acid, fructose, sorbitol, xylitol and stevia on the daily biomass growth of opportunistic microorganisms 71-76

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Introduction. The use of probiotic bacteria as biotransformator system is a promising way to obtain new derivatives of known substances with antimicrobial or other beneficial activity. The aim of the research was to investigate the effect of the *Bifidobacterim bifidum* cell-free supernatants, obtained by cultivation of bifidobacteria in their own disintegrate supplemented with ascorbic acid (BbAsc), fructose (BbFr), sorbitol (BbSor), xylitol (BbXyl) or stevia (BbSt) and the substances themselves (Asc, Sor, Xyl or St) on the growth of opportunistic microorganisms. **Material & methods.** The effect of the studied supernatants and substances on the daily biomass growth of the test cultures was investigated by spectrophotometry using a 96-well polystyrene microtiter plates and a «LisaScanEM» spectrophotometer («ErbaLachemas.r.o.», Czech Republic). Reference strains *Staphylococcus aureus* ATCC 25923; *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as a test cultures. The final concentration of the studied supernatants and substances in the incubation medium was 30%vol, and the final concentration of bacterial cells was ~10^6 CFU/ml. Inhibition (II) or stimulation (SI) indices of the daily biomass growth of test cultures were calculated by the formula $II\ (SI) = \frac{\Delta Dt - \Delta Dc}{\Delta Dc} \times 100\%$, where ΔDt and ΔDc are the optical density gain of test and control samples. **Results & discussion.** Among the studied substances, stevia alone did not affect the daily biomass growth of any of the test cultures, either as in solution with a final concentration of 30 mg/ml or as in a supernatant of *B. bifidum* culture. Fructose at a final concentration of 30 mg/ml had the same effect on test cultures growth as the supernatant of *B. bifidum* culture after probiotic cultivation with fructose: it inhibited staphylococcal growth (Fr: II = 30,9 %, BbFr: II = 34,6 %), stimulated the growth of *P. aeruginosa* (Fr: IS = 23,7 %, BbFr: IS = 19,8 %) and did not affect the growth of *E. coli*. Xylitol at a final concentration of 30 mg/ml did not affect the growth of *P. aeruginosa* biomass, but inhibited the growth of *E. coli* (II = 20,9 %) and *S. aureus* (II = 28,4 %). The supernatant of

B. bifidum, cultured in the presence of xylitol inhibited the growth of *S. aureus* biomass (II = 26,1 %) and did not affect the growth of the other test cultures. The sorbitol and cell-free supernatant of *B. bifidum* cultured in the presence of this polyol equally influenced the biomass growth of test cultures: had no significant effect on the daily biomass growth of *S. aureus* but stimulated the growth of *E. coli* (BbSor: IS = 43,9 %, Sor: IS = 34,5 %) and *P. aeruginosa* (BbSor: IS = 26,4 %, Sor: IS = 28 %). The only substance that significantly inhibited the biomass growth of all test cultures was ascorbic acid. Cell-free supernatant of *B. bifidum* (BbAsc) cultured in disintegrate supplemented with ascorbic acid caused more pronounced inhibition of the daily biomass growth (*S. aureus* – 78 %, *E. coli* – 52 % and *P. aeruginosa* – 45 %) compared to ascorbic acid (Asc) itself (*S. aureus* – 42 %, *E. coli* – 35 % and *P. aeruginosa* – 38 %). **Conclusion.** Among the studied substances, only ascorbic acid caused significant inhibition of biomass growth of all test cultures. *B. bifidum* sell-free supernatant obtained by cultivation of bifidobacteria in their own disintegrate supplemented with ascorbic acid caused a more severe inhibition of test cultures growth than ascorbic acid itself. The absence of signs of chemical modification of ascorbic acid in the *B. bifidum* cell-free supernatant on the chromatogram indicates that the greater inhibitory effect of this supernatant is due to the synergistic effect of ascorbic acid and the inhibitory compounds produced by *B. bifidum* during cultivation. The other studied substances showed various effects on the daily biomass growth of test cultures. The use of *B. bifidum* as a biotransformer system, and xylitol, sorbitol, fructose and stevia as precursors for the production of new antimicrobials by combinatorial biosynthesis, turned out to be not effective enough.
Keywords: *Bifidobacterim bifidum*, cell-free supernatants, ascorbic acid, fructose, sorbitol, xylitol stevia, biomass growth, opportunistic microorganisms

Clinical and microbiological studies of aminoglycosides efficiency in purulent inflammatory processes 77-85
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Introduction. Infectious complications of diabetic foot syndrome are one of the main complications of diabetes mellitus, as well as a significant risk factor for amputation of the lower limb. The use of effective antimicrobial therapy is an important component in the treatment of these infections. Knowing the problem of antibiotic resistance in our time, the choice of starting antimicrobial drug is very important to reduce ineffective treatment, resistance to antibacterial agents, unwanted complications and economic costs. **Objective.** Conduct a comparative microbiological study of the antimicrobial efficacy of aminoglycosides against pathogens of wound suppurative-inflammatory processes and determine the clinical effectiveness of these agents. **Materials and methods.** The study included 45 patients with purulent-inflammatory processes of wounds in diabetic foot syndrome who received surgical treatment and antibiotic therapy. Patients were randomly assigned to three groups according to the prescription of antibiotics from the class of aminoglycosides (tobramycin, amikacin, gentamicin). Clinically took into account the general condition of the patients, wound healing, laboratory parameters (blood test, procalcitonin, CRP). Microbiological research was carried out in the bacteriological laboratory of the Department of Microbiology. Microbiological identification of the isolated microorganisms was carried out by the classical method according to morphological, tinctorial, cultural, biochemical features. **Results & discussion**. Microbiologically established polymicrobiality of wound contents in patients with diabetic foot Syndrome. Gram-positive and gram-negative microorganisms were identified. Aminoglycosides (gentamicin, tobramycin, amikacin) provide the same bacteriostatic, bactericidal effect on sensitive clinical strains of S. aureus, S. epidermidis, E. coli, E. cloacae, P. aeruginosa, K. teriggena, tobramycin had the advantage of antimicrobial activity to A baumannii, E. faecalis, and resistant strains of staphylococcus ($p < 0.05$). As a result of studies, good tolerance by patients to antibiotics from the aminoglycoside group as starting monotherapy in 91.8% was revealed. according to A. baumannii, E. faecalis, and resistant staphylococcus strains ($p < 0.05$). Clinically, tobramycin also showed advantages in local wound healing and, according to laboratory data (a decrease in the level of leukocytosis, procalcitonin and CRP), is 30% more effective in the first 6 days of antibiotic therapy, compared with group 2 and 3 of the study. **Conclusion.** Considering the polymicrobial spectrum of infectious pathogens in diabetic foot syndrome and the antibiotic resistance of the use of aminoglycosides in monotherapy for mild to moderate processes and in combination therapy with other antibiotics for severe degrees of complications, this is a balanced strategy for preventing the development of microbial resistance. Aminoglycosides (gentamicin, tobramycin, amikacin) provide both bacteriostatic and bactericidal effects on gram-positive and gram-negative pathogens, and especially tobramycin, which has the advantages of antimicrobial activity against A. baumannii, E. faecalis, and resistant strains of staphylococcus.
Keywords: diabetic foot syndrome, gram-positive pathogens, gram-negative pathogens, antibiotics, aminoglycosides.

Bioinformation analysis of polymorphism of the *blaTEM* gene coding regions in *Escherichia coli* strains 86-89
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Bioinformation analysis of the bacterial genome with screening of genetic elements to detect variability of nucleotide sequences is one of the methods of antibiotic resistance evolution fundamental research. Comparative analysis of the molecular genetic variability or genome sequence conservatism is used as a convenient tool for understanding patterns of the evolutionary process through definition of the phylogenetic relationships. **The aim of the study** was to conduct a bioinformation analysis of the variability of the antibiotic resistance *blaTEM* gene in *E. coli* strains. **Materials and methods.** The GenBank nucleic acid search database was used for bioinformation analysis. The search for nucleotide sequences of the *blaTEM* gene was performed in fasta-format. "Vector NTI Advance 11.0" software package was used to analyze the nucleotide sequences of the *blaTEM* gene. Multiple alignment and its statistical analysis to determine conservative and variable regions of the *blaTEM* gene was performed using software component module "AlignX". Nucleotide sequences of the 21 *blaTEM* gene sequences isolated from *E. coli* strains in 11 countries and registered in the GenBank database from 1996 to 2019 were analyzed. **Research results and discussion.** Multiple alignment of the nucleotide sequences of the studied *blaTEM* gene sequences allowed to determine both conservative and variable regions of the gene. 12 conservative regions of the *blaTEM* gene with a length of at least 30 nucleotide sequences were identified. The longest homologous region of the gene (144 nucleotide sequences) was found at the 529 - 672 nn position. *blaTEM* gene variability was evidenced by point mutations at 41 positions of the nucleotide sequences. The most heterogeneous region was found at the 697-717 nn and 773-813 nn positions. Mutations in the analyzed *E. coli blaTEM* genes were caused by transitions (60,5%), transversions (37,2%) and deletions (2,3%). The degree of relatedness of the analyzed *blaTEM* gene sequences is presented in the form of a dendrogram. The phylogenetic tree shows three conditional genetic clusters. The top position of the dendrogram is occupied by the cluster that includes eight *blaTEM* gene sequences, isolated in European countries: 2 - in France and one each - in Poland, Germany, England, Portugal, Italy and the Netherlands. This cluster was characterized by a significant number of evolutionary events (from 4 to 9) associated with nucleotide sequence substitutions, which is represented on the dendrogram by the divergence of the phylogenetic tree branches. The cluster which occupies the middle position of the dendrogram is represented by five phylogenetic branches. This cluster includes *blaTEM* gene sequences isolated in Korea, China, Germany and India. 1-2 nucleotide substitutions were identified in the majority of the sequences in this cluster. The lower position of the dendrogram consists of *blaTEM* gene sequences characterized by a high degree of relatedness and a small number of nucleotide sequence substitutions, a significant number of sequences were isolated in the United States and China (75%). **Conclusions.** Results of the bioinformation analysis showed that the nucleotide sequences of the *E. coli blaTEM* gene have historically undergone several evolutionary divergences and acquired signs of heterogeneity, which apparently causes the emergence of a large number of species of the *blaTEM* gene**.**
Key words: bioinformation analysis, *E. coli*, *blaTEM* gene.

Introduction. The most effective way to combat infectious diseases is to vaccinate the population. Each country develops its own vaccination schedule, which takes into account the specifics of the epidemic situation, the availability of registered vaccines, financial opportunities and other factors. Experimental and clinical studies have shown that vaccines can cause both suppression and activation of individual immune functions, with each vaccine having its own spectrum of effects on the quantitative and functional characteristics of the immune status. There is a need to correct the formation of immunity during vaccination, taking into account factors affecting the intensity of a specific response to vaccine administration. It is proposed to use the principles of individualization of vaccination (revaccination), primarily in high-risk groups, which include people with persistent herpes virus infection. **Material & methods.** An immunological examination was carried out, including determination of the concentration of cytokines TNFα, IL10, IFNγ, levels of CD3 + CD4 + and CD3 + CD8 +, levels of subclasses 1 and 3 of specific IgG antibodies, viral load (HVL). The study material was blood in a volume of 3.0-5.0 ml, which was taken from a vein in compliance with the usual rules of asepsis. To determine the concentration of TNFα, IL10, IFNγ cytokines in blood serum, the Vector-Best ELISA test systems were used: Gamma-interferon IFA-BEST, interleukin-10 - IFA-BEST, Alpha-TNF IFA-BEST. Herpesviridae family antigens (Ag) were determined by immunofluorescence using specific monoclonal mouse antibodies from Santa Cruz Biotechnologu, Inc. (USA) and viral load (herpes viral load, HVL). CD3 + CD4 + and CD3 + CD8 + were studied by flow cytometry on a CYTOMICSFC500 (Beckman Coulter, USA) using the MKAT panel (Beckman Coulter, USA). Subclasses of specific IgG antibodies are determined by ELISA in modification. In this case, 96-well panels coated with antigens are used, respectively, according to a commercial kit for the determination of specific IgG antibodies (Euroimmun or Human, Germany). Serum is added at a 1:50 dilution. The conjugate is peroxidase-labeled anti-IgG1, IgG2, IgG3 and IgG4 monoclonal antibodies ("Polygnost") at a concentration of 1 μg / ml [9]. Calculation formulas for determining the dynamics of the complex integral indicator over time were obtained from the initial data for the determination of the levels of specific vaccination antibodies and complex integral indicator using mathematical modeling of linear and non-linear functions of the exponential distribution. **Results & discussion.** According to the results of studies, we propose to determine the ratio of such immunological parameters: specific IgG1sp / IgG3sp; immunoregulatory index CD4 / CD8; TNFα / IL10, IFNγ / IL10 ratio and herpes virus load HVL (herpes viral load); Using the formula IgG1sp / IgG3sp * CD4 / CD8 * 0.1 * TNFα / IL10 * 0.01 * IFNγ / IL10 * HVL (c.u.), the complex integral index (CII) is calculated. The probability of a decrease in the post-vaccination immunity of the subject is judged by the change in the complex integral indicator over time (according to the chart). **Conclusion.** The development of a new method for determining the probability of a decrease in post-vaccination immunity in individuals with herpesvirus load is aimed at increasing the assessment of the body's immunity against controlled infections and helps to decide on the need for revaccination or its delay for a certain period of time, which is calculated individually. The end result is a reduction in the incidence of infectious diseases.
Keywords: herpes virus, postvaccination effects, immunity

For more than a century, the name of I.I. Mechnikov has been shining in the constellation of the names of outstanding scientists, whose works laid the foundations for the formation of a scientific worldview of all living things: from a small microorganism to a person. He is considered the founder of comparative pathology and the theory of immunity, a scientist who stood at the origins of evolutionary embryology, microbiology and immunology. He formulated the general theory of inflammation as a protective reaction of the body in the fight against infection. The enormous significance of Mechnikov's scientific heritage is based primarily on deep materialistic and evolutionary principles, a consistent conductor and passionate champion of which he has been throughout his life. Biology and medicine owe the scientist not only many brilliant discoveries and firmly facts, but also significant broad generalizations laid the foundation for a number of the most progressive areas of modern biology and medicine. These include the concept of «intravascular inflammation». Using this definition, Mechnikov argued about the possibility of phagocytosis of bacteria by circulating leukocytes. The main idea formulated by him is consonant with the modern concept that, according to I. I. Mechnikov, «harmful factors», getting into the blood, excite the reaction of phagocytes, the main effector mechanism of inflammation. What happens with the classic inflammatory response is «extravasal», thanks to the emigration of cells from the bloodstream. He speaks of «intravascular inflammation» only once, without focusing attention, however, he testifies to the correspondence with the main idea. The scientific thought formulated by Mechnikov, rethought and reinforced by the results of studies of his followers, found its continuation in the modern concept of intravascular inflammation. In general, it can be argued that Mechnikov made a scientific feat, the scale of which becomes all the more obvious the farther he is from us in the historical interval and there is no doubt that the ideas and methodology of our great countryman will be key in the 21st century for new fundamental discoveries in the field of natural sciences.
Keywords: Intravascular inflammation, I. I.Mechnikov, history, immunity

Our compatriot, well-known scientist, Nobel Prize winner in medicine and physiology Ilya Ilyich Mechnikov left a bright mark not only in domestic but also in world science. Ilya Ilyich's multifaceted scientific activity was devoted to various areas of research in biology and medicine. The scientist's success in studying important problems of zoology, embryology, comparative pathology, gerontology, immunology, virology and bacteriology is renowned around the world. One of the areas of his research activity aimed at studying tuberculosis. In May 1911, the Pasteur Institute organized the Astrakhan expedition led by I.I. Mechnikov. Leading scientists were among the participants of the expedition – specialists in microbiology and epidemiology from Russia, France, Italy and Japan. The expedition was to address important issues related to the spread of plague and tuberculosis in the Kalmyk steppes. Ilya Ilyich Mechnikov set the task to use diagnostic tests (Pirke test and ophthalmological test) to determine the level of tuberculosis infection in the steppe population and to investigate the relationship between the incidence of tuberculosis and increased contact of Kalmyks with non-steppe population. The work of the Astrakhan expedition led by I.I. Mechnikov became the starting point for successful studies of tuberculosis infection. When beginning studying tuberculosis, I.I. Mechnikov planned to completely defeat this disease. Although he did not achieve this goal, scientific conclusions made by Mechnikov while studying biological properties of the pathogen and mechanisms of immunity to tuberculosis were further developed, and joint efforts of domestic and foreign scientists made it possible to elaborate tailor-made measures for particular regions to prevent tuberculosis and justify the use of antimicrobials in the treatment of this infection.
Keywords: I.I. Mechnikov, Astrakhan expedition, tuberculosis.