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OPTIMIZATION OF EXPRESSION, PURIFICATION, AND STABILIZATION CONDITIONS FOR FLG-HA2-4M2E, A RECOMBINANT PROTEIN IN UNIVERSAL INFLUENZA VACCINE

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The aim of this work was to increase the yield of Flg-HA2-4M2e recombinant protein, which is the main component of a broadly protective (universal) intranasal influenza vaccine. Flg-HA2-4M2e includes a hemagglutinin stalk (aa76-130) consensus fragment of influenza A viruses belonging to phylogenetic group 2 (HA2-2) joined with 4 tandem copies of M2e (human influenza viruses A M2 protein ectodomain). Those fragments were sequentially linked to the C-terminus of flagellin and a 6-histidine tag was added to the N-terminus. The sequence was cloned into pQE30, transformed into *E. coli* DLT1270, and cells were grown in LB medium at 37°C. When an OD₆₀₀ of ~0.5–0.7 was reached, the culture was cooled rapidly and IPTG (1 mM final) was added. During optimization, the expression temperature was reduced from 37 to 28°C and the duration was increased from 4 to 18 hrs. Because of these changes, we obtained the protein in soluble form, thus avoiding refolding during further purification. Cells were collected by centrifugation and frozen at –20°C overnight, it was not destroyed immediately as before. The lysis method was changed from lysozyme to sonication. Densitometry showed that the level of expression increased from ~5 to ~25% of total protein. Protein purification was carried out using metal affinity chromatography with a Ni-sorbent. Due to the protein's expression in soluble form, it was purified using native buffers (without urea). Column elution was carried out using a linear imidazole gradient, which yielded a cleaner product than stepwise elution. Flg-HA2-4M2e was evaluated by SDS-PAGE, which indicated a single band (*MW*~74 kDa) of ~95% purity. Western Blot, using antibodies specific to flagellin and M2e, confirmed the presence of those proteins. Protein stabilization conditions were compared; L-arginine, Tween-80, sucrose, and polyglukin were tried as stabilizers. L-arginine was chosen according to the results of densitometry, and the stability of the protein during 4 months of storage was verified by SDS-PAGE.

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THE ROLE OF MORAXELLA CATARRHALIS IN THE DEVELOPMENT OF COMPLICATIONS AFTER INFLUENZA AND OTHER ACUTE RESPIRATORY DISEASES

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Annually in the world from flu and its complications die from 200 to 500 thousand people. However, it is not possible to identify the causative agent of community-acquired pneumonia in 50–60% of patients. According to domestic authors, 0 to 15% of cases of acute respiratory diseases (ARD) are etiologically associated with *M. ca-*

tarrhalis, while in foreign publications *M. catarrhalis* is associated with 20 to 35% of cases. Cause of low detection of strains *M. catarrhalis* is a laborious cultural method of isolating microorganisms and the lack of regulatory documents for the study of genetic and phenotypic markers of bacterial virulence. The aim of the study: to determine the role of *M. catarrhalis* in the development of complications of influenza and other ARD.

The study involved 339 patients aged 18 to 48 years with influenza and other acute respiratory infections, of which 299 patients with complications. The control group consisted of 320 healthy individuals. The methods used were bacteriological, virological, mass spectrometric analysis, methods of detection of genetic and phenotypic markers of virulence, methods of statistical analysis.

It was found that the most significant in the structure of bacterial complications in patients with influenza and ARD are community-acquired pneumonia (29% of cases) caused by *M. catarrhalis* (31%), which is present in monoculture and in combination with gram-positive coccal flora (38%). We studied the genetic and phenotypic markers of virulence in *M. catarrhalis* strains isolated from patients with angina, bronchitis, sinusitis and pneumonia for the presence of the *mcaP* gene that encodes the production of McaP protein. He takes part in adhesion of *Moraxella* to the cells of the mucous epithelium. As a control, we investigated *M. catarrhalis* strains isolated from healthy individuals. It was shown that in 84% of cases *M. catarrhalis* strains isolated from patients had the *mcaP* gene, while in healthy individuals bacteria had it in 14% of cases. In addition, the etiological role of *M. catarrhalis* strains in the development of pneumonia was proved by the presence of the highest adhesion index to buccal epithelial cells (17.3±2.7) in contrast to (4.5±0.6) in strains isolated from healthy individuals.

In the case of isolation of *M. catarrhalis* from patients with complicated flu and other ARD, it is necessary to carry out genetic typing of strains to detect the gene of virulence of *mcaP*. In phenotypic confirmation of *mcaP* gene expression, it is necessary to consider this pathogen as an etiological factor of the disease.

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THE IMPORTANCE OF PATHOGENICITY FACTORS OF SOME SPECIES OF STAPHYLOCOCCUS, STREPTOCOCCUS AND KLEBSIELLA IN DETERMINING THEIR ETIOLOGICAL ROLE IN THE DEVELOPMENT OF INFLAMMATORY PROCESSES OF THE RESPIRATORY TRACT

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Currently, the greatest difficulty in the diagnosis of infectious diseases of the respiratory tract is to determine the etiological role of the isolated microorganisms, especially if they belong to the group of opportunistic or kommensals. More than half of these diseases remain unencrypted, because they are allocated conditionally pathogenic bacteria are not subject to etiological accounting because of species. The aim of the study was to characterize the virulence of opportunistic bacterial infectious disease of the respiratory tract and to improve the methodology of the etiological decryption.

We studied 100 strains of *Staphylococcus epidermidis*, 220 strains of *Streptococcus* spp., 125 strains of *Klebsiella*

spp. We used the following methods: bacteriological methods of isolation of microorganisms from clinical material, methods of detection of phenotypic virulence markers, methods of detection of virulence genes, mathematical methods of data processing.

The result revealed that the strains of *S. epidermidis* isolated from patients with chronic rhinitis and sinusitis had the virulence gene *icaA* responsible for adhesive properties, 5 times more frequently than in strains isolated from healthy individuals. In the phenotypic test in buccal cells, the strains of *S. epidermidis* had an index of adhesion is 3 times higher than strains isolated from healthy individuals. Some species of *Streptococcus* such as *S. mitis*, *S. anginosus*, *S. oralis*, isolated from patients with high frequency. In the study of strains of these species for the presence of virulence genes *sagA*, *lmb*, *fapI*, *ply*, *lytA* was found that the strains isolated from patients, were often several times than the strains isolated from healthy individuals. The adhesion index of strains isolated from patients were 2–4 times higher than that of strains isolated from healthy individuals. Strains of *Klebsiella oxytoca* isolated from humans with sinusitis, had virulence genes *MrkD*, *magA*, *kfu*, which are characteristic for strains of *Klebsiella pneumoniae*. In phenotypic tests it was found that the adhesion index in *K. oxytoca* strains isolated from patients was 4 times higher than in strains of this species isolated from healthy individuals. To confirm the etiological role of the opportunistic microorganism in the development of the infectious process, it is necessary to be guided by data on genetic and phenotypic markers of virulence of the isolated strain. This will help to prescribe adequate therapy in the early stages of the infectious process and prevent the development of complications.

3.30 doi: 10.15789/2220-7619-2018-4-3.30 PARVOVIRUS INFECTION: DISSEMINATION, MEDICAL-SOCIAL SIGNIFICANCE

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The clinical importance of parvovirus infection (PVI) is associated with the teratogenic effect of the virus, as well as its ability to disrupt erythropoiesis. Currently, in a number of countries, including Russia, there is no systematic registration of PVI cases, therefore the true extent of its spread is unknown. Also, the problems are not solved of preventing congenital PVI in children, as well as of viral safety at transfusion of blood and its components. However, the detection of cases of PVI in the Russian Federation has increased significantly during the rubella integration into the measles elimination program, which involves a laboratory examination of patients with exanthematous diseases. Identification of cases of PVI on the territories of the North-West Federal District (NWFD) of the Russian Federation in the period 2014–2017 allows to obtain new information about the spread of infection in the region. 1044 blood sera of patients with maculopapular rash and fever were received from 11 territories of the NWFD. The presence of serum IgM antibodies to parvovirus B19 was assessed as sign of acute PVI. Specific IgM antibodies were detected using the Anti-Parvovirus B19 ELISA IgM test system (EUROIMMUN, Germany). IgG antibodies were used to examine 733 sera from clinically healthy men and women aged 18 to 60 years.

The assay was performed with the Anti-Parvovirus B19 ELISA IgG test system (EUROIMMUN, Germany). It has been established that PVI is continuously identified in 10 of the 11 districts of the region, mainly in the areas close to borders. The prevalence was observed among patients of children of 3–6 years (25.3% of cases) and 7–14 years (33.3% of cases). A high proportion of seropositive among the examined donors was found (75.4–88.9%). On the contrary, a low proportion of seropositive (56.7%) pregnant women was detected. A high proportion of errors in the primary diagnosis of PVI is shown. The main erroneous clinical diagnosis was “rubella”.

These results are in agreement with those obtained earlier (2009–2012) and indicate a wide spread of PVI in the NWFD. Population immunity is actively formed in organized collectives. In the main risk group (pregnant women), the proportion of people who are sensitive to infection is high (43.3%). At the stage of elimination of measles and rubella, it is necessary to carry out differential diagnosis with parvovirus infection in each case of exanthematous disease.

3.31 doi: 10.15789/2220-7619-2018-4-3.31 SENSITIVITY OF 2018 KAZAKHSTAN INFLUENZA TYPE B VIRUSES TO ANTIVIRAL DRUGS

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As it's known, a characteristic feature of the influenza virus is its high variability, manifested by a wide variety of properties of circulating seasonal strains. One of the characteristics of epidemically relevant viruses which is most important to clinical practice is their sensitivity to chemotherapy drugs.

To examine the drug sensitivity of three influenza type B viruses isolated in 2018 from clinical samples collected in health care facilities located in Almaty region, etiotropic anti-influenza drugs, including Remantadine, Tamiflu, Arbidol and Ingavirin, were used. The experiments were carried out on chick embryos. The resistance of influenza viruses to different concentrations of antiviral drugs was assessed by the level of reproductive suppression of 100 infectious doses for the virus. The dose of the drug that suppressed twofold the virus titer in the hemagglutination reaction compared to the control was considered as the inhibitory concentration (IC_{50}).

The Kazakhstan influenza B viruses showed a high degree of sensitivity to Tamiflu (IC_{50} values were 0.58 to 1.40 mg/mL). The strains studied were less sensitive to Remantadine since their reproduction was inhibited by the drug at concentrations of 13.13–37.50 mg/mL. The isolates exhibited absolute stability in relation to the drug Ingavirin, except for one virus, which showed a low degree of sensitivity, because it was suppressed to less than 50% when using the highest dose of the drug (50 mg/mL). The strains taken in the experiment turned out to be completely resistant to Arbidol.

The performed experiments thereby showed that the examined 2018 Kazakhstan isolates of influenza type B virus are sensitive to Tamiflu and Remantadine and resistant to Arbidol and Ingavirin.