3.27 doi: 10.15789/2220-7619-2018-4-3.27

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 phylogenic 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 OD_{600} 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.

Supported by: Russian Science Foundation (Project No. 15-14-00043-П).

3.28 doi: 10.15789/2220-7619-2018-4-3.28

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. catarrhalis*, 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.

3.29 doi: 10.15789/2220-7619-2018-4-3.29

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*