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THE USE OF RAPD-PCR FOR GENOTYPING OF *S.* ENTERITIDIS ISOLATED DURING THE OUTBREAK

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For a long time *Salmonella enterica* serovar Enteritidis remains the main pathogen of salmonellosis in Russia. Traditionally, different typing methods are used for outbreak investigation to establish the identity of isolates, to identify the way and factors of transmission. Despite of all their advantages, pulse filed gel electrophoresis (PFGE) and whole genome sequencing, which are the "gold typing standards", are currently unavailable for the majority of the bacteriological laboratories because of their complexity and high requirements for the staff qualification.

The aim of this work was to assess the possibility to use the RAPD-PCR for the rapid genotyping of *S.* Enteritidis strains isolated during outbreaks. 27 strains of *S.* Enteritidis isolated from patients involved in two unrelated *Salmonella* outbreaks were studied. 13 strains were isolated during the first outbreak, 14 strains — during the second. For RAPD-PCR four oligonucleotide primers were used: mnv-45, mnv-3-1, mnv-3-2 and RAPD4. Amplified fragments were separated by electrophoresis in a 1.5% agarose gel in 0.5 x TBE-buffer. Analysis of electrophoresis pictures was carried out using Bio-Rad Universal Hood II Gel Doc System.

All experiments with *Salmonella* strains were performed in three repeats to confirm the result reproducibility. Primer mnv-3-2 had the best discriminating ability and allowed to separate the strains belonging to different outbreaks by the number and length of amplified fragments. Primers mnv-3-1, mnv-45 and RAPD4 gave the almost identical profiles of amplified fragments, and mnv-45 also required a special electrophoretic conditions, since the amplified fragments differed slightly (by 20–30 base pairs).

The obtained results allow us to recommend the use of RAPD-PCR method with mnv-3-2 primer for operative genotyping of *S.* Enteritidis isolates, which reveals the individual characteristics of epidemic strains. If the laboratory is equipped with PCR equipment with electrophoretic detection, the strain typing can be performed within one working day.

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A NEW VISION AT THE IDENTIFICATION OF THERMOTOLERANT CAMPYLOBACTERS

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Earlier in the bacteriological laboratories the identification has always been considered as the easiest step in Campylobacter diagnostic, since thermotolerant Campylobacters have a typical morphology, if the strains have been isolated on special media and in the microaerobic atmosphere. It allowed to use only two key tests for differentiation of *C. jejuni*, *C. coli* and *C. lari*: hippurate hydrolysis and indoxyl acetate.

In case of impossibility to carry out the species identification, it was enough to establish that the isolate belonged to thermotolerant *Campylobacter*s, since all of them were considered as etiological agents of the acute diarrhea.

Currently, the discovery of new types of *Campylobacters* (*C. avium* in 2009 and *C. hepaticus* 2016) requires a revision of the approach to the identification of thermotolerant *Campylobacters*. They are also thermotolerant and capable of hippurates hydrolysis like *C. jejuni*. The pathogenicity of *C. avium* is not identified, and *C. hepaticus* cause the spotty liver disease of chickens, but do not cause disease in humans. Currently known ecological features of *C. avium* made it unlikely their frequent isolation in the routine laboratory practice. As concern of *C. hepaticus*, there can be a problem with the interpretation of the results during the examination of both clinical material and food products.

The emergence of new *Campylobacter* species requires the revision of phenotypic identification algorithms and extensive use of previously little used tests: the reduction of selenite and nitrates, the growth in the presence of 2.3,5 TTC, the hemolytic activity. Commercial test systems (API Campy) do not currently allow to identify *C. avium* and *C. hepaticus*. It is also necessary to update the databases of MALDI TOF mass spectrometry and to develop the specific primers for identification the new types of *Campylobacter* in PCR.

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IDENTIFICATION OF ROTAVIRUS I- AND E-GENOTYPES BY MULTIPLEX PCR METHOD

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In recent years, the sudden appearance of reassortant rotaviruses was reported in many countries (Australia, Hungary, Vietnam, Spain, Thailand, Japan). Their rate sometimes amounted to 46.7%. These strains cannot be detected using the binary classification (G[P]-genotype) only. Identification of reassortants is possible with complementary analysis of gene segments encoding the inner capsid protein VP6 and enterotoxin NSP4. The aim of this work was the development of multiplex PCR methods for determination of rotavirus I (VP6) and E (NSP4) genotypes.

Rotavirus-positive fecal samples from children accommodated with acute gastroenteritis in the infectious hospital of Nizhny Novgorod were used. The nucleotide sequences of the VP6 and NSP4 genes were determined on the Beckman Coulter CEQTM 8000 (Beckman Coulter, USA). The phylogenetic analysis was carried out using the BEAST 1.8 software package. MEGA 6.0, UGENE, and OligoCalc were used for the primers design.

At the first step, the variety of rotaviruses in Nizhny Novgorod was studied on the basis of the VP6 and NSP4 genes of 16 strains. Two I-genotypes (I1 and I2) and three E-genotypes (E1, E2 and E3) were determined. Three alleles of the VP6 gene (I1-1, I2-IV, and I2-VII) and four ones of the NSP4 gene (E1-I, E2-X, E2-XII, and E3) were shown. Additionally, 128 nucleotide sequences of the VP6 and NSP4 genes of rotaviruses from other 23 countries were also analyzed. Next, sequences of reverse primers specific for the most common among human rotaviruses I- and E-genotypes were selected. In combination with the forward primers, they flanked the regions of the VP6 and NSP4 genes with lengths of 195 bp (I3), 273 bp (I1), 368 bp (I2) and 233 bp (E3), 305 bp (E2), 443 bp (E1), respectively, which could be detected by agarose gel electrophoresis. The optimal annealing temperatures for primers

(57°C and 55°C) and the concentration of magnesium ions in the reaction mixes (2.5 mM) were determined. The PCR conditions and primers specificity were tested using a sample of 16 previously characterized specimens. The results of genotyping by the developed methods fully corresponded to the data of sequencing.

Thus, these methods can be used for routine determination of I- and E-genotypes in rotavirus-positive samples and for identification of reassortant rotavirus strains.

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CXCL10 GENE PROMOTER POLYMORPHISM A-1447G MODULATES PROTEIN EXPRESSION IN SERUM AND ASSOCIATED WITH INVASIVE ASPERGILLOSIS IN FEMALE ONCOHEMATOLOGICAL PATIENTS

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Invasive aspergillosis (IA) — is life-threatening invasive infection, especially in immunocompromised hosts, most of which are oncogematological patients. The key components of fungal infections pathogenesis are disturbances of the immune system. Chemokine CXCL10, also known as interferon gamma-induced protein 10 (IP10), is a member of CXC chemokines. CXCL10 is an inflammatory mediator, which stimulates the directional migration of Th1 cells as well as increasing T-cell adhesion to endothelium. *CXCL10* gene promoter single nucleotide polymorphisms (SNPs) affects protein expression via NF-κB transactivation.

The purpose of this study is to investigate of allelic variants A-1447G (rs 4508917) and G-135A (rs 56061981) effect on the amount of CXCL10 protein in serum and risk of development IA in oncohematological patients in St. Petersburg.

171 oncogematological patients on the background of cytostatic polychemotherapy with symptoms of lung injury were recruited to participate this study. 75 oncogematological patients (44.5%) either developed proven or probable IA as defined by criteria of EORTC/MSG 2008 (median age 43±14, 57% males) whereas controls (96 oncogematological patients (55.5%) without IA comparable in age and sex) did not fulfill these criteria.

SNPs was analyzed by the method of restriction fragment length polymorphism analysis. Chemokine CXCL10 amount was determined with the use of commercial ELISA kit sets (Cloud-Clone Corp, USA). Statistical analysis was performed using SPSS 21 (IBM, USA).

The heterozygous AG rs 4508917 and homozygous GG rs 56061981 genotypes prevailed in the both studied groups and there were no significant differences in genotype distribution of A-1447G and G-135A between oncogematological patients with probable IA and without IAL. However, when dividing patients by sex in a female group G allele rs 4508917 was significant associated with the occurrence of IA ($\chi^2 = 3.853$, $p < 0.50$, OR 3.13 95% CI (1.196–8.204).

There were no differences in serum CXCL10 levels between –135 GG and GA genotypes. However, individuals with –1447G allele had significantly higher serum levels of CXCL10 than those with –1447(A/A) genotype ($p = 0.022$).

Further increase in the number of patients included in the study will allow to make conclusions about the prospect of typing the studied polymorphic variant of the gene CXCL10 as a predictive marker of the risk of mycosis development with a strong significance.

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DEVELOPMENT OF A PANEL OF MONOCLONAL ANTIBODIES FOR STUDYING OF LOCAL PRODUCTION OF CYTOKINES IN CHRONIC RHINOSINUSITIS

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Cytokines as key regulators of inflammation play a central role in the pathophysiology of chronic rhinosinusitis (CRS). CRSs are divided into CRS with and without polyps of the nasal mucosa, but this difference is not sufficient for a clear definition of subgroups with the same pathophysiology and production of cytokines. This area remains open for more detailed studies. The purpose of our work was the development of a panel of monoclonal antibodies for studying the characteristics of local production of cytokines in CRS. For studies, monoclonal antibodies to human cytokines were obtained using hybridoma technologies. One or more clones producing antibodies to cytokines (IL-1β, IL-4, IL-6, IL-8, IL-17, TNFα, GM-CSF, IFNα, IFNγ) were obtained. Specificity of antibodies was proved in ELISA: direct and sandwich method. To create a panel, the antibodies were tested by indirect immunohistochemistry using the avidin-biotin-alkaline phosphatase system. Isolated peripheral blood mononuclear cells from three donors were stimulated LPS 500 μg/ml or PHA 20 μg/ml overnight at +37°C at 5% CO_2.The cell smears on the glasses were fixed with 4% PF. The antibodies studied were used as the first antibodies, dilutions were selected in preliminary experiments. Under the optical microscope, the numbers of lymphocytes or monocyte having red staining in the cytoplasm were counted; the result was expressed as a percentage. The reaction intensity was expressed in points (1-moderate, 2-medium, 3-intensive reaction). As a result, clones were selected that produce antibodies that best detect cytokines in human cells. After induction LPS, IL-1β was detected in 41.0±19.3% of lymphocytes (intensity 2 points) and 90.7±1.3% of monocytes (3 points); IL-6 in 4.0±1.53% of lymphocytes (2 points) and 78.7±8.4% of monocytes (2–3 points), IL-8 in 10.0±4.6% of lymphocytes 1–2 points) and 48.0±10.6% monocytes (1–2 points); TNFα was rarely detected in lymphocytes, in 46.7±18.9% of monocytes (1–3 points); IFNα was detected mainly in monocytes (77.7±10.0%, 2–3 points); The weak but distinct GM-CSF production was determined in 56.7±18.6% monocytes (1–2 points). After PHA induction IL-4 was detected in 6.0±2.5% of lymphocytes (1–2 points), 47.3±2.0 monocytes (1–2 points); IL-17 — was determined in 38.67±15.4% monocytes (1–2 points); IFNγ — in 16.7±11.6% of lymphocytes (1 point) and 32.3±6.38% of monocytes (1–2 points). Thus, it was shown that the obtained antibodies reliably detect the corresponding cytokines in human cells. This panel of antibodies will be used by us to assess the specific features of local production of cytokines in CRS, as well as a number of other inflammatory processes.