58m strain (GenBank Acc. No. LGAN00000000) genome was detected. Also blaOXA-335 beta-lactamase gene was identified in the genome of the strain *A. lwoffii* 51m (Acc. No. LZDF00000000) from mammoth intestinal tract (Goncharov A. et al., 2016). Based on this data we believe that the formation of epidemic clones in opportunistic bacteria is more likely determined by the natural selection of strains that carry genetic determinants of pathogenicity or resistance to antimicrobial drugs from natural populations (including in the polar regions of our planet), than by their formation in human society *de novo*.

2.6 doi: 10.15789/2220-7619-2018-4-2.6

THE RESULTS OF THE RESEARCH OF USING A COMMERCIAL KIT FOR DETENTION THE RABIES VIRUS`S RNA IN THE COURSE OF EVALUATION OF THE INFECTION OF THE FIELD MATERIAL

E.A. Gradoboeva[1], E.M. Poleshchuk[2], A.A. Pogoda[3], G.N. Sidorov[2], I.V. Deriglazov[4]

[1]Omsk State Medical University, Omsk, Russia; [2]Omsk Research Institute of Natural Focal Infections, Omsk, Russia; [3]Ltd "Fractal Bio", St. Petersburg, Russia; [4]Centre of Hygiene and epidemiology in Omsk Region, Omsk, Russia

There are 3000 animal diseases of the Rabies registered annually in Russia (Poleshchuk et al., 2013). The monitoring of this disease helps to reduce it for animals and people. Forward-looking molecular genetic methods are used more and more often in order to diagnose the disease (Iovleva et al., 2012; Devyatkin et al., 2014; Dedkov et al., 2016).

"The kit for the detection of the Rabies virus`s RNA in a complete set" produced by Ltd "Fractal Bio" (St. Petersburg) is a set of reagents for the outflow of the nucleic acid and the realization of the real-time PCR with hybridization-fluorescent registration, it was used to analyze the Rabies of 210 samples: 153 field material and 57 samples of bioprobes.

The initial material included samples of foxes, racoon dogs, corsac foxes, minks, Siberian striped weasels, martens, ferrets, sables, ermines, bats and men as a 10% suspension of a cerebrum based on Henx solution. There were also the samples of bioprobes of not purebred white mice that died in the course of the experiment. The samples were collected from 2–11 passages and were presented by 10% suspensions of the cerebrum. The apparatus used during the research was RotorGene6000.

With the use of test-system, the presence of Rabies virus`s RNA was confirmed in all positive samples of the initial material of foxes (n = 14), cattle (n = 6) and men (n = 2). None was detected in all the negative samples. If negative probes, they registered the formation in the reaction of the product of the amplification of the internal control sample (genomic DNA of mammals).

A different selection of the field material revealed drawbacks of the set when using the internal control sample (ICS). Therefore, ICS did not work with the initial samples of racoon dogs (n = 10), bats (n = 12) and all the mice samples. When researching the initial material of foxes (n = 35) and corsac foxes (n = 3), there was no reaction of the ICS or it was very weak (from the 30^{th} — 36^{th} cycle) in more than 50% cases. The dilute of the nucleic acid, repeated several times, did not improve the result of the reaction with the ICS. That means it lacks the universality of the amplifiable fragment. Provided that there is high reliability of identification of the Rabies virus`s RNA, the negative results of the ICS make the interpretation of the result more difficult.

Therefore, the above-mentioned test-system needs refining in part of the optimization of the ICS.

2.7 doi: 10.15789/2220-7619-2018-4-2.7

COLLISION OF CRISPR-CAS SYSTEMS WITH THE POTENTIAL OF VIRULENCE OF *ESCHERICHIA COLI* STRAINS THAT PRODUCE SHIGA-TOXINS

E.I. Ivanova[1], N.A. Arefieva[2], Yu.P. Dzhioev[3], T.V. Tunik[1], A.Yu. Borisenko[3], L.A. Stepanenko[3], E.A. Kungurtseva[1], E.V. Grigorova[1], U.M. Nemchenko[1], L.V. Rychkova[1]

[1]Scientific Center for Family Health and Human Reproduction Problems, Irkutsk, Russia; [2]Irkutsk State University, Irkutsk, Russia; [3]Research Institute for Biomedical Technologies of Irkutsk State Medical University, Irkutsk, Russia

Shiga toxin-producing *Escherichia coli* (STEC) strains are a diverse group of *E. coli* strains belonging to over 400 *E. coli* serotypes, some of which cause outbreaks and sporadic cases of food-borne illnesses ranging from diarrhea to hemorrhagic colitis and the hemolytic-uremic syndrome (HUS). It was long believed that bacteria could not resist phage attacks, but in 1987 a strange region was discovered in the *E. coli* genome that consisted of multiple repeats. The discovered structures were termed CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats — CRISPR-associated proteins). CRISPR is a chronological record of infectious assault on a bacterium from viral and other genetic elements. According to Delannoy S. et al., It has been shown that CRISPR polymorphisms in *E. coli* strongly correlate with both the serotype of the microorganism and the presence of virulence factors in its genome (*stx* and *eae* genes).

Genomic sequences (GenBank databases) of *E. coli* isolates of different serogroups (n = 658) were analyzed for the presence of CRISPR-Cas systems and stx-genes. Of the 658 *E. coli* isolates, 60.5% of the loci of CRISPR-Cas systems were found. At the same time, according to the structural organization of CRISPR-Cas, the systems of the strains studied were of type I-E in 92.7% and type I-F of 7.3%. Analysis for the presence of genes of shiga toxins 1 and 2 types showed that 14.4% of isolates having a CRISPR-Cas system of type I-E were positive. The genes *stx1A* and *stx1B* were registered in 6.1 and 5.9% of cases. The frequency of registration of *stx2* subtypes was 2 times higher than *stx1* (6.1 and 12.3%). *Stx2B* was detected in 9.9% of cases.

Stx2A, which according to the literature is more often associated with HUS than other subtypes, was detected in 9.7%. According to the CRISPR-Cas data, the I-E subtype system is associated with the *stx1A* (10.1%), *stx1B* (9.8%) *stx2A* (16.1%) and *stx2B* (16.3%) genes. Further linkage between CRISPR elements and the pathogenicity of the isolate will allow us to determine the causal relationships that stimulate the acquisition of the isolate of both the CRISPR-Cas system and the genes coding for pathogenicity.

2.8 doi: 10.15789/2220-7619-2018-4-2.8

NGS CAPABILITIES FOR THE STUDY OF ENTEROAGGREGATIVE *E. COLI*

A.A. Kechin[1,2], D.S. Boldyreva[1,2], M.A. Makarova[3], L.A. Kaftyreva[3], S.G. Scherbak[4,5], S.V. Apalko[5], M.L. Filipenko[1,2], A.M. Sarana[4,5], M.A. Churina[5,6]

[1]Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia; [2]Novosibirsk State University, Novosibirsk, Russia; [3]St. Petersburg Pasteur Institute, St. Petersburg, Russia; [4]St. Petersburg State University, St. Petersburg, Russia; [5]City Hospital No. 40 of the Resort Administrative District, St. Petersburg, Russia; [6]St. Petersburg Clinical Infectious Diseases Hospital named after S.P. Botkin, St. Petersburg, Russia

Enteroaggregative *E. coli* (EAggEC) are the causative agents of such intestinal diseases as acute and chronic diarrhea, inflammation of the intestine in children and

adults, and growth deficiency in children. EAggEC are among the most frequent pathogens of nosocomial infections, often leading to the death of patients. EAggEC isolates are characterized by the presence of a wide range of virulence factors and resistance to the standard spectrum of antibiotics.

The goal of this work was to determine possibilities of the massive-parallel sequencing (NGS) for the entoreaggregative *E. coli* study in comparison with standard *in vitro* tests.

We examined 8 strains of *E. coli* isolated from children under 2 years of age with the diagnosis of "intestinal dysbiosis". Determination of sequences of isolate genomes was carried out using massive parallel sequencing on a MiSeq (Illumina) instrument using MiSeq reagent kit v2 reagents (500 cycles).

Phenotypic resistance to antibiotics was determined by the disc-diffusion method. The detection of drug resistance genes in the resulting genomic sequences was carried out by the ResFinder program. Virulence factors were determined by identifying the virulence genes EAggEC by PCR in a multiplex format followed by electrophoretic detection, as well as the VirulenceFinder software, which searches for the corresponding sequences in the genomic sequences.

Most often, the isolates were resistant to β-lactams (6 isolates), aminoglycosides (6 isolates) and sulfonamides (5 isolates). In most cases (29 of 34), the results obtained from the analysis of the full genomic sequencing data coincided with the results of the disco diffusion test, even in cases of a low average coverage of the reference genome, which indicates the applicability of NGS for the study of bacterial isolates for resistance to antibacterial drugs.

The VirulenceFinder program on average found 12 (from 5 to 18) virulence factors for each isolate. The most common genes encoding virulence factors, such as *aap* (encodes dispersin, 7 isolates) and ORF4 (6 isolates). Genes coding for toxins, *astA* and *sat*, met in the genomes of 3 and 2 isolates, respectively. When compared with PCR data, the results were the same in 88% of cases (35 of 40).

Moreover, analysis of NGS data gave information on genome structure, MLST and serotype phenotypes. Thus, NGS can extend results of *in vitro* tests of enteroaggregative *E. coli* without loss of other significant information.

2.9 doi: 10.15789/2220-7619-2018-4-2.9

ALGORITHM OF EXPRESS LABORATORY DIAGNOSTICS IN THE STUDY OF DIPHTHERIA

G.N. Khamdulaeva[1], L.A. Kraeva[1,2], G.I. Bespalova[3]

[1]St. Petersburg Pasteur Institute, St. Petersburg, Russia; [2]S.M. Kirov Military Medical Academy, St. Petersburg, Russia; [3]North-Western State Medical University named after I.I. Mechnikov, St. Petersburg, Russia

In recent years, against the background of active migration processes, negative attitude to vaccination in part of the population, as well as the loss of attention to some infections, there is a risk of foci and the spread of diphtheria. In Western Europe, diphtheria cases are associated with the importation of infection from Africa and the Middle East. In Russia, migration flows are directed from the countries of Central Asia, which border on the regions with high incidence of diphtheria (the countries of the Indian Peninsula). Therefore, the aim of the work was to develop a method for rapid and effective screening for diphtheria and to create an algorithm for laboratory diagnosis.

The study used bacteriological, molecular, mass spectrometric (MALDI-TOF/MS) methods and technology "lab on a chip". The method was tested on 400 strains of *Corynebacterium* of different species, including 180 strains of *Corynebacterium diphtheriae*.

As a result, we have developed a method of rapid growth of bacteria and their identification in 3 hours. The biological sample is sown on a porous membrane with a pore diameter of 5 μm filled with agarose gel according to an improved formulation. This makes it possible to grow all the bacteria at once in a "clean" culture, and, in just 3 hours. Next, the video sensor registers the image of the emerging colonies of bacteria, and a special computer program processes the images and determines the type of microbes. The specificity of this method in determining the genus of bacteria is 95%, in determining the species — 85%.

The development of an express method of cultivation and identification of bacteria allowed the authors to propose an algorithm for rapid laboratory diagnosis of diphtheria. Previously, A. Berger et al. proposed an algorithm that allows to obtain a result on the presence of a toxigenic strain of *C. diphtheriae* in a biological sample at least 48 hours. Algorithm offered by us allows to identify the bacteria *C. diphtheriae* and determine their toxicity using previously presented techniques for 3.5 hours. This will allow rapid and qualitative screening of large groups of the population, including migration centers, for diphtheria, which will help to identify the patient or carrier of *C. diphtheriae* in time and prevent the spread of infection.

2.10 doi: 10.15789/2220-7619-2018-4-2.10

MODERN LABORATORY DIAGNOSTICS OF ESCHERICHIOSIS

M.A. Makarova, Z.N. Matveeva, L.A. Kaftyreva

St. Petersburg Pasteur Institute, St. Petersburg, Russia

For many years, for differentiation of *E. coli* phenotypic methods based on biochemical identification and O-serotyping (agglutination with antiserum) were used. But these methods are insufficient as the majority of biochemical properties and serogroups are common for both pathogenic and non-pathogenic *E. coli*. Currently the molecular methods allow obtaining useful information about O- and H-antigens, virulence genes and other genetic markers.

400 strains of *E.coli* isolated in 2014–2017 were investigated. The strains belonged to five serogroups: O1 (200 strains), O144 (125), O26 (52), O111 (17) and O55 (6), and were official registered as the pathogens of acute enteric infections.

In *E. coli* O1, the antigenic formula O1:K1:H7 was determined by molecular serotyping. The strains hadn't virulence genes of diarrheagenic *E.coli* but had the genes typical for ExPEC *(pap, sfa, hly, cnf, aer)*. According to the data of literature, the strains with this antigenic formula and virulence genes are highly virulent for birds, and are capable of causing UTI in human.

The strains of *E. coli* O144, isolated from healthy people but officially registered as EIEC, belonged to the biovar 2 and hadn't invasive genes *(ial, ipaH)*.

According to modern data, *E. coli* of serological groups O26, 55 and 111 can refer to two pathogens: EPEC and EHEC, and are subject for epidemiological surveillance, since EHEC infection can occur with HUS and renal failure. The molecular serotyping has showed that all strains of *E. coli* O26 belonged to the same serovar