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

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ALGORITHM OF EXPRESS LABORATORY DIAGNOSTICS IN THE STUDY OF DIPHTHERIA

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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

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MODERN LABORATORY DIAGNOSTICS OF ESCHERICHIOSIS

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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

O26:H11 (*rfb*O26, *fliC*11); *E. coli* O111 — to 2 serovars: O111:H8 (5 strains *rfb*O111, *fliC*8) and O111:H2 (12 strains of *rfb*O111, *fliC*2). *E. coli* O55 also belonged to 2 serovars: O55:H7 (one strain, *rfb*O55, *fliC*7) and O55:H6 (five strains of *rfb*O55, *fliC*6). According to our data, 10 strains of O26:H11, 5 strains of O111:H8 and 1 strain of O55:H7 had *stx1* gene (encoding the production of shiga-like toxin 1) in combination with *eae* gene (the adhesion factor, intimin) and could be consider as EHEC. All strains *E. coli* O111:H2 and *E. coli* O55: H6 and 42 *E. coli* O26: H11 had only *eae* gene, indicating that these strains belonged to the EPEC.

The introduction of molecular methods of serotyping and detection of virulence factors in laboratory diagnostics makes it possible to confirm the true pathogenicity of *E. coli* strains and to minimize the diagnostic errors in etiological interpretation of acute enteric infections.

2.11

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COMPARISON OF PHENOTYPIC AND MOLECULAR-GENETIC PROPERTIES OF THE STRAINS *NEISSERIA MENINGITIDIS* ISOLATED FROM PATIENTS WITH GENERALIZED FORMS OF MENINGOCOCCAL INFECTION AND CARRIERS

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Characterization of isolates of *Neisseria meningitidis* obtained from patients with meningococcal disease or from nasopharyngeal swabs of asymptomatic carriers can be achieved by several methods which provide different levels of discrimination.

A total of 42 gram-negative, oxidase-positive diplococcus strains isolated from individuals with meningococcal disease in 2009–2018 years and 65 isolates from 1075 nasopharyngeal carriers in 2016–2018 years were examined by three approaches: serological typing by agglutination, determination of the serogroups by real-time PCR, multilocus sequence typing (MLST). Each strain from patients with meningococcal disease was also determined sensitivity to antibiotics by dilution in broth.

The majority of strains isolated from patients belonged according to the results of agglutination and real-time PCR data to serogroup B (50 and 40.5% respectively), C (16.7 and 11.9% respectively) and W (14.3% by results of both methods). Among the isolates from carriers according to the results of agglutination and real-time PCR data were dominated serogroup W (37.0 and 30.8% respectively) and B (32.3 and 26.2% respectively). Invasive isolates of serogroup B were resistant to penicillin (28.6%), levofloxacin (33.3%), chloramphenicol (27.3%), rifampicin (14.3%), invasive isolates of serogroup W135 to chloramphenicol (9.1%).

MLST established the genetic relationships of the isolates from patients and identified members of known hypervirulent lineage CC11 (n = 4).

Six isolates of *N. meningitidis* (invasive and from nasopharyngeal swab) were additionally investigated by whole genome sequencing.

The results are included in the GenBank international database: SRR7352647 SAMN09435696 Nm-146 blood 16-Apr-2018, SRR7352646 SAMN09435695 Nm-105 nasopharyngeal swab Aug-2016 (https://www.ncbi.nlm.nih.gov/sra/SRP150714)

2.12

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MOLECULAR TYPING IN RESEARCH OF EPIDEMICAL CHOLERA MANIFESTATION

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The causative agent of El Tor cholera evolved adaptive mechanisms to ensure its' preservation and accumulation in certain ecological niches and provide existence of its population in various climatic and geographical zones. Considering this, epidemics development mechanism differs in endemic and non-endemic areas. Siberian and the Far East regions are non-endemic territories for cholera. The last epidemic complications in this region were reported in 1990s and had a form of certain infection importation cases and acute outbreaks, associated with importations. Along with this, strains of the *Vibrio cholerae* El Tor, devoid of the pathogenicity determinants, are found annually in environmental objects.

The aim of this work is to elucidate the patterns of cholera epidemiological manifestations in Siberia and the Far East, based on a complex molecular genetic analysis of *V. cholerae* El Tor strains.

In complex assay, using amplification profiling, MLVA-, PFGE-, MLST-, and wgSNP-typing, we found, that *V. cholerae* El Tor strains isolated in epidemic complications, homogenous in basic pathogenicity, pandemicity, persistence determinants along with nucleotide context of housekeeping genes, are characterized by diversity in the associated with pathogenicity genomic loci structure, macrorestriction patterns, SNP-profiles, and structure of variable tandem repeats loci. At the same time, closely related subclones of one clonal variant were identified within the individual outbreaks. Considering the typing data, we can conclude that the outbreaks genesis in the non-endemic territories of the region is determined by the hyperinfectious clone importation. Circulation of closely related subclonal vibrio variants during the outbreak can be caused by environmental factors during the implementation of the water or the contact-household transmission routes.

V. cholerae O1 El Tor isolates from surface watercourse in a cholera free period are characterized by a significant polymorphism of MLVA profiles and PFGE genotypes; that indicates a high heterogeneity of the water populations of the microorganism and the probability of microevolutionary changes during persistence in surface watercourse, as well as the possible periodic introduction of new clones into aquatic ecosystems.

Thus, molecular approaches in the analysis of cholera manifestations provides an understanding of the epidemic complications development patterns in the territory and *V. cholerae* persistence in the environment.

2.13

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PCR FOR DIAGNOSIS OF GONOCOCCAL INFECTION: PANACEA OR ESCAPE FROM REALITY

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The aim of this study was to provide an analytical assessment of the role and place of PCR in gonococcal infection (GI) monitoring. Clinical guidelines define a set of laboratory tests for its diagnosis, including molecular genetic techniques (MGT). It is considered, that MGT have the highest diagnostic sensitivity, in contrast to the traditional procedures (microscopic, bacteriolo-