

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 enteroaggregative *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  $\beta$ -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

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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  $\mu$ m 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

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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