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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