

traintestinal diseases, sometimes leading to morbidity and mortality. The aim of this work was to study of genetic diversity and assess of pathogenic potential of commensal *E. coli* isolated from healthy adults in Saint Petersburg. 300 *E. coli* strains were collected from fecal samples of 50 St. Petersburg's inhabitants. *E. coli* strains were isolated using Endo agar and identified by biochemical tests. Determination of four major phylogenetic groups and identification of virulence genes were performed by using real-time, multiplex and simplex PCR. Seven genes typical for ExPEC (*fimH*, *pap*, *sfa*, *aer*, *afa*, *cnf1*, *hlyA*) were identified among the analyzed strains. The B2 phylogroup (47.1%) was leading among other groups: A (20.5%), B1 (9.0%) and D (23.4%). Each strain had at least one virulence gene. No strain had all seven studied genes simultaneously. The maximum number of genes in one strain was five. The prevalence of virulence genes was as follows: *fimH* (98.0%), *pap* (25.0%), *sfa* (8.0%), *aer* (33.8%), *afa* (5.6%), *cnf1* (11.0%), *hlyA* (10.0%). The strains of groups B2 and D harbored the virulence determinants significantly more frequently than the strains of groups A and B1. Our results showed that *E. coli* isolated from adults differ in their phylogenetic structure and harbour a greater variety of virulence genes. Our study revealed that commensal *E. coli* isolated from healthy humans constitute a substantial reservoir of genes related to the extraintestinal pathotypes. All seven tested virulence genes typical for ExPEC were detected and it's important that the prevalence of these genes was significantly higher among the isolates from healthy adults. So, the extraintestinal virulence genes (encoding the adhesins, toxins, persistence) were found not only in pathogens, but also in commensal microflora of healthy people. Previous reports indicated that virulence genes associated with extraintestinal pathogenesis in fact help the *E. coli* strains to colonize the human gut; therefore, they may be considered as a fitness factor and the virulence is a coincidental side effect.

9.18

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EXPRESS METHOD OF GROWING BACTERIA ON THE MEMBRANE OF ANODIC ALUMINIUM OXIDE

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The classical method of studying bacteria is the cultivation of microorganisms and study of their biological properties. However, this method is very long (several days). So it may not be used in cases when you need to quickly get the result. It can be surgery, sepsis, severe infection, etc. In these cases, the doctor will need a few hours to make a decision on the appointment of causal treatment.

We have developed a method of growing the isolated clones of bacteria from any biological material for 3 hours. The rapid growth of microorganisms is ensured due to the new culture medium. Each microbial cell is grown in a separate cell on a porous membrane of anodic aluminum oxide. After 3 hours of incubation reads visual information using a specially developed image sensor zoom. The visual image of the individual microcolony identified to the species created by special computer programs. The probability of coincidence of the results is 90%. With the help of a special counter counts the number of bacteria of each species in the studied sample. This is especially important in the study of biological material containing several types of microorganisms.

Thus, 3 hours after inoculation of biological material, we get the result about of species and quantitative composition of bacteria. A living culture of microorganisms can work with it further to explore other biological properties, including rapid determination of sensitivity to antibiotics.

9.19

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THE DRUG RESISTANCE MUTATIONS OF THE HEPATITIS B VIRUS AMONG HIV-INFECTED INDIVIDUALS

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Hepatitis B virus (HBV) is one of the most common hepatotropic viruses that can cause both acute and chronic course of the disease. One form of chronic viral hepatitis B is occult hepatitis B, characterized by the presence of HBV DNA in the liver and undetectable levels of HBsAg and HBV DNA in the peripheral blood. The co-infection of HBV with the human immunodeficiency virus (HIV) is facilitated by the common mechanisms and pathways of infection. Although the effect of HBV on the progression of HIV infection appears to be minimal, HIV affects the progression of liver fibrosis, increasing the risk of developing hepatocellular carcinoma and cirrhosis. The need for timely identification HBV variants carrying drug resistance mutations among HBV/HIV-coinfected patients.

The aim of our study was to evaluate the prevalence of HBV with drug resistance mutations among HBV/HIV-coinfected patients.

The material was blood plasma of 264 HIV-infected (HBsAg-) patients with virologic ineffectiveness of ARVT. A method for detecting HBV DNA with a low viral load based on a two-step PCR, followed by sequencing was used.

HBV DNA was detected in 89 (33.7%) patients. Based on the phylogenetic analysis it was shown that in this group the HBV subgenotypes are represented in the following ratios: D1 — 39.3%, D2 — 29.2%, D3 — 30.4%, C1 — 1.1%, respectively. In the analysis of nucleotide sequences in the viral polymerase reverse transcriptase domain significant amino acid substitutions (mutations described in the literature as determining the development of drug resistance to nucleotide/nucleoside analogues therapy) were found in 12.35% of patients. Including 9 patient was found to have significant amino-acid replacement in HBV polymerase gene (L180M, M204V) associated with the development of resistance to lamivudine, entecavir, telbivudine and tenofovir. Also in 5.6% of patients were found potentially significant (substitutions in the same significant positions of the polymerase gene, but not described in the literature) — for example L80F.

The obtained data on the prevalence of HBV drug resistance indicate the need for screening of patients with HBV/HIV-coinfection before starting the antiviral therapy.

9.20

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INCREASE OF SELECTIVE AND GROWTH PROPERTIES OF A NUTRIENT MEDIUM FOR IDENTIFICATION AND ACCUMULATION TRICHOMONAS VAGINALIS

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Currently, the detection of patients with trichomoniasis is decreasing. This effect can be caused by asymptomatic infection, imperfect methods of protozoa identification, low availability and quality of nutrient media. Therefore,

the improvement of selective diagnostic nutrient media for the detection of *T. vaginalis* is so important.

The purpose of the study was to increase the selectivity and growth properties of the nutrient medium for the *T. vaginalis* detection. It was necessary to select a concentration of amphotericin B in media with different content of horse serum and peptone enzymatic, which inhibits the growth of *Candida* spp., but doesn't affect the growth of *T. vaginalis*.

For the research, an experimental mediums (based on the SVT medium (RU FSS No. 2009/05982), produced by the Pasteur Institute) were prepared with different content of horse serum (10%, 20%, 40%), half of which were with enzyme peptone (12.8 g/l) and all of them without antimycotics. Two dilutions of amphotericin B in the range of concentrations of 0.5–50 µg/ml, as well as fluconazole at a concentration of 264 mg/ml (as in the SVT) were introduced into all experimental media. The strains of *T. vaginalis* (T1, T5, T7, T11) from the Pasteur Institute collection in concentration 0.5×10^6 cells/ml and the standard strain *Candida albicans* ATCC 24433 in concentration 10^7 cells/ml were sown in all medias in three recurrence. The incubation temperature was $35 \pm 1^\circ\text{C}$. Counting the number of cells was carried out using the Goryaev chamber every day for a week. After 24 hours, *C. albicans* were sowed onto the Müller–Hinton agar for testing the suppression of their viability with subsequent microscopy.

It was revealed, that optimal accumulation of *T. vaginalis* (2.5×10^6 cells/ml) and inhibition of *C. albicans* occurs at concentration of amphotericin B (2–0.5) µg/ml. The activity of fluconazole to *C. albicans* in these media was low. It should be noted, that in medias with a high content of horse serum, the accumulation of *T. vaginalis* increased sharply on the second day of the study, and their resistance to high concentrations of antimycotic (up to 20 µg/ml) was also observed. However, the viability of the cells was reduced in contrast to media with 10% horse serum and low concentrations of amphotericin B. The addition of enzymatic peptone to experimental media did not reveal a significant difference in the growth properties.

According to the results, an experimental medium containing 10% horse serum with amphotericin B in concentration 2 µg/ml was chosen for detection and accumulate *T. vaginalis*.

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ANALYSIS OF THE PHAGE SENSITIVITY OF MICROORGANISMS OF A MICROBIOTA OF A VAGINA

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Recently in connection with growth of detection of antibiotic resistant cultures, for treatment of infectious diseases even more often recommend to use bacteriophages. Bacteriophages don't give side effects in comparison with antibiotics and work is specific on microorganisms and exist in different pharmaceutical forms: liquid, gel and tableted. The solution of a question of application of a phage has to be based on results of testing of activity of medicine.

The aim of the study was comparative analysis of two options of phagus medicines for definition of a phagus sensitive of microorganisms of a microbiota of a vagina.

50 women who have addressed to laboratory on an outpatient basis for the purpose of receiving a bacteriological research of a vaginal microbiota have been examined. Bacteriological researches were conducted according to the standard recommendations. For identification of species of bacteria by MALDI-TOF MS method used a desktop mass spectrometer of Microflex with the MALDI Biotyper library (Bruker Daltonics Germany). As the tested medicines applied polyvalent liquid and gel forms of bacteriophages. The modified technique where the bacteriophage was applied with a print on culture a bacteriological loop (cm d = 0.5) bent at an angle of 90° lehas been developed for a gel form of a bacteriophage. Assessment of lytic activity of a phage was carried out on a five-point scale (by quantity of "crosses").

The sensitivity to bacteriophages has been defined at 45 women with violation of a microbiota of V. At the same time the high sensitivity to a liquid form of a bacteriophage has been found in 6 patients (13%). To a gel form the high sensitivity has been defined at 39 patients (87%), coincidence cases on sensitivity at both bacteriophages weren't observed.

The parallel research of sensitivity of microflora to liquid and gel forms at a bacterial vaginosis has shown that in 87% microorganisms were sensitive to a gel form while the sensitivity to liquid bacteriophages has been found in 13% of the bacteria inhabiting the offered modification of a research of activity of bacteriophages on a gel basis allows to dose bacteriophages in this pharmaceutical form and to receive comparable results with a classical technique.

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S. AUREUS/C. ALBICANS MONO- AND DUAL-SPECIES BIOFILMS

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Several studies have reported the co-isolation of *S. aureus* and *C. albicans* from numerous biofilm-associated diseases. These data indicate that these organisms have the capacity to interact with one another at the molecular level. The possibility of the development of polymicrobial biofilms, consisting of both fungi and bacteria, should be considered in pathogenesis of various infections.

The aim of our work was study of mono- and dual-species *S. aureus/C. albicans* biofilms, evaluation of distinctions between clinical and standard streins biofilms of both in static assays.

In the work used standard strains of *S. aureus* 25923 ATCC and *C. albicans* CCM 885, as well as clinical strains of them, isolated from patients with acute otitis media (*S. aureus* U14) and also from healthy carrier (*S. aureus* 609, *C. albicans* 609).

Overnight cultures was diluted 1:100 into fresh medium for biofilm assays. Static biofilm assays of O'Toole G. and Kolter R. (1998) in a 96 well dish was used. Biofilms were formed by adding both organisms 1:1 to either microtiter plates. The plate was then grown statically at 37°C overnight. The cultures removed with a multichannel pipette plate, the plate was rinsed 3–4 times with water, a 0.1% solution of crystal violet in water was added of to each well, incubated for 15 minutes at RT. Then 200 µL of 95% ethanol was added to each well and the plate was left to stand on the bench for 30 minutes. Finally the plate was read with a microplate reader Multiskan at 620 nm.

Biofilms density of *Staphylococcus* cultures was the highest in the clinical isolate *S. aureus* U14 (24% more