Improvement of methods of microbiological diagnosis of mixed infections in women of reproductive age

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Abstract. *Isolation of Listeria monocytogenes from clinical material in obstetric-gynecological pathology is often difficult due to the presence of associate microbes. This study presents materials from the study of new domestic nutrient media for the isolation of listeria in the microbiological diagnosis of listeriosis in pregnant women. The diagnostic efficiency of the developed nutrient media in laboratory diagnostics of mixed infections in women with obstetric and gynecological pathology is shown.*

Keywords: *Listeria monocytogenes, listeriosis of pregnant women, isolation, culture media, microbes-associates, inhibition*

Relevance

In recent years, more and more attention of researchers and doctors of various specialties is attracted by opportunistic infections, the problem of diagnostics of which has not yet been resolved. The causative agents of such infections fully include listeria, which cause the development of listeriosis, a disease characterized by a wide range of clinical manifestations (2, 6).

Listeria infection poses the greatest danger to pregnant women and newborns, causing miscarriages, stillbirths, and fetal malformations. In most cases, due to the late diagnosis of listeriosis in pregnant women, transplacental infection of the fetus is observed, and, according to average data, 22% of pregnancy ends in abortion or stillbirth (6,7). Premature birth in these cases is common, as is the development of the disease in newborns. At the same time, the study of clinical material when symptoms of listeriosis appear in pregnant women would reveal its true share in the structure of abortion. For Listeria, as for other facultative intracellular parasites, the main role is played by cellular immunity, a decrease in which during pregnancy increases the susceptibility to listeria infection in patients of this risk group (3, 6, 7).

The polymorphism of clinical manifestations, as well as the frequent combination of L. monocytogenes with other microorganisms, significantly complicates the clinical diagnosis of listeria infection; therefore, the priority in this area is the bacteriological research method using selective culture media. Sowing on solid nutrient media ("Gold Standard") is still the leading research method that allows you to isolate, identify and study the causative agent of infection (1, 4, 6, 8).

The aim of this work is to study the diagnostic efficiency of domestic culture media and various laboratory research methods in the microbiological diagnosis of perinatal listeriosis.

Materials and methods

The material for the study was smears and scrapings from the cervical canal, taken in compliance with all the rules for taking material for research, which were studied by bacteriological, serological methods, as well as by PCR diagnostics (1, 4, 5).

Comprehensive examination included mandatory smear microscopy according to the generally accepted method (Gram stain) and quantitative inoculation on an expanded set of nutrient media with cultivation under aerobic conditions for (18±2) h at a temperature of (37±1)°C.

652 pregnant women who were registered in the infertility rooms of the antenatal clinics of Makhachkala were examined. All women were of active reproductive age (20-35 years). Of these, 60% of the examined were diagnosed with secondary infertility with recurrent miscarriages (58%) and stillbirth (42%). In 40% of the examined women, the following diagnoses were established: cervical erosion (46%), colpitis (19%), endometritis (15%), vaginitis (7%) and vaginal candidiasis (13%). All women in this group had an obstetric history of miscarriage.

To detect L. monocytogenes in clinical material, domestic culture media were used for the accumulation, isolation and identification of listeria (FSUE Microgen NPO Nutrient media, Makhachkala).

Considering that, as a rule, the accompanying microflora in obstetric and gynecological infections is represented by bacteria of the Enterobacteriaceae genus, such as E. coli, Klebsiella spp, Proteus spp, as well as Candida albicans, the growth of which on the developed selective media for Listeria was completely suppressed, for the isolation of these microorganisms In parallel, the well-known commercial media Endo, Levin, Candida-agar, ampholan-agar (for the isolation of Proteus) were used, which made it possible to shorten the time of bacteriological research and accelerate the process of identifying the causative agent of the infection in the examined group of pregnant women.

Results and discussion

The results of the study of the clinical material showed that in most cases a mixed microflora was found, represented by combinations of listeria with microorganisms of various natures (bacteria, fungi, viruses).

In all cases of clinical manifestation of listeria infection, when inoculated on a nutrient medium for accumulation, the broth was cloudy; on the medium for the isolation of listeria, the growth of small colorless colonies in the S-form, shiny, bluish when examined in transmitted light, was observed. On the medium for the primary identification of Listeria, after (18±2) h, the growth of black colonies, shiny, moist in the S-form, d = 1.0±0.2 mm was revealed.

In the examined groups of women, 24 strains of L. monocytogenes (21.4%) were isolated and identified to species, of which stillbirth was diagnosed in 14 cases, and miscarriage was diagnosed in 10 cases. All of them had a history of 2 or more miscarriages in the long term of pregnancy (21-25 weeks).

The results of serological studies showed that all 24 isolated pathogens belonged to L. monocytogenes serogroups 4b and 1/2a, and gave an agglutination reaction for "+++" or "++++".

The belonging of 24 isolates to the species L. monocytogenes was confirmed by polymerase chain reaction (PCR) with species-specific primers. The rest of the genus Listeria tested negative for PCR.

The isolated strains of Listeria were tested to determine their sensitivity to antibacterial drugs. Antibiotic sensitivity was determined by the disk diffusion method. We used commercial antibiotic discs (manufactured by NICF, St. Petersburg). The antibioticogram of Listeria was studied by inoculating the isolated cultures of L. monocytogenes (2.0 ml of inoculum from a standard dilution of 10^9) on AGV nutrient medium to determine sensitivity to antibiotics (produced by NPO Nutrient Media). In the experiment were taken antibiotics most often used in the treatment of listeriosis. The results of the antibiogram showed that one hundred listeria was highly sensitive to linezolid (MIC was 60.0 μg/ml) and rifampicin; sensitive to penicillin, vancomycin, ampicillin, gentamicin and erythromycin; resistant to nalidixic acid.

Among the representatives of the accompanying bacterial flora, E. coli was most often detected - 41.8%, microorganisms of the genus Klebsiella spp. - in 28.7%, S. faecalis - in 4.5% and fungi of the genus Candida - in 5.1%. Bacteria of other genera were found less frequently.

Most of the isolated cultures caused hemolysis of erythrocytes (on blood agar), especially often this feature was noted in L. monocytogenes (95%), in E. coli (70.0%), P. vulgaris (28.0%), S. marcescens (25.0%), Klebsiella spp. (50%), Staphylococcus spp. (25%), P. mirabilis (75.0%). The test for DNase in E. coli was positive in 50.0%, in S. marcescens in 100%, less often DNA-ase was found in bacteria of the genus Klebsiella spp. (in 10.0%).

Lecithinase activity was detected in 30.0% of Proteus strains. Hyaluronidase was produced by almost all isolated opportunistic representatives of the enterobacteriaceae family, for example, bacteria of the genera Klebsiella, Proteus in 100% of cases.

Clinical DNA samples isolated from scrapings of epithelial cells of the cervical canal of women with diagnoses of miscarriage and stillbirth were analyzed for the presence of specific fragments using PCR-Ureaplasma urealiticum, Mycoplasma hominis and genitalium, Chlamydia trachomatis, Trichomonas vaginalis, and 2 titles), HPV (16-18 titles).

The detection of causative agents of these infections by the PCR method was different, so in 37% of cases chlamydia, Trichomonas - 21%, human papilloma virus - 18%, gonococcus - 11%, ureaplasma - 8%, herpes simplex virus - 5% were detected. Studies have shown that of the listed viruses, the greatest etiological role in the development of mixed infections in the surveyed group of women is played by chlamydia (37%).

Conclusions

Thus, it can be concluded that the developed media for the isolation and cultivation of Listeria had a high sensitivity and specificity in relation to the growth of these pathogens, and also ensured the stable preservation of their basic properties. Listeria isolated from the material under study were typical in their morphological and biological properties: gram-positive rods; catalase-positive; had mobility at 22°C; disposed of esculin; fermented with the formation of

acid rhamnose and mannose and did not ferment beckon, xylose and arabinose; formed a ß-hemolysis zone on 5% blood agar; exhibited lecithinase activity in the presence of activated carbon and yolk emulsion. The results of studies of clinical material showed that listeria and opportunistic enterobacteria, isolated using the developed media, along with specific infections in obstetric and gynecological pathology in the examined groups of women, had a number of factors that determine the possibility of their participation in the pathogenesis of diseases. The data of bacteriological studies were confirmed by PCR-diagnostics methods and in the reaction of indirect immunofluorescence, the percentage of coincidences when using 3 methods was 98-100%.

References

1. Bakulov I.A., Vasiliev D.A. Listeriosis as a foodborne infection. The issue of diagnosis and prevention. Ulyanovsk, 1991.

2. Dolgikh T.I. laboratory diagnostics – the basis for information support of the diagnostic process in opportunistic infections.

3. Ermolaeva S.A., Bely Yu.F., Tartakovsky I.S. Changes in the expression level of L. monocytogenes virulence factors under the influence of external conditions. Molecular Genetic Microbiology and Virology, 2000; 1: 17-19.

4. Karpova T.I., Ermolaeva, Lopyrev I.V., Brodinova N.S., Tartakovsky I.S., Vasquez-Boland H.A. New methods for the identification of Listeria monocytogenes. Clin. Microbiol. antimicrobial. chemother. 2001; 1: 80-81.

5. Karpova T.I., Firsova T.E., Rodina L.V., Kotlyarov V.M., Tartakovsky I.S., Ermolaeva S.A. Typing of Listeria monocytogenes based on gene polymorphism of pathogenicity factors. Clin. Microbiol. antimicrobial. chemother. 2003; 3: 251-258.

6. Omarova S.M. Microbiological diagnosis of listeria infection (textbook). Makhachkala, 2018, P. 40.

7. Omarova S.M., Isaeva R.I., Akhmedova R.S. Microbiological aspects of listeria infection in pregnant women and newborns. Makhachkala. 2017, P.63.

8. Tartakovsky I.S., Maleev V.V., Ermolaeva S.A. Listeria: role in human infectious pathology and laboratory diagnosis. M., "Medicine for All", 2002.