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FERTILIZATION FAILURE IN HEIFERS INFECTED BY UREAPLASMA DIVERSUM

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Ureaplasma diversum is an opportunistic pathogen in cattle, but colonization of the respiratory tract by this ureaplasma, and its carriage in the reproductive tract may lead to the serious diseases. It also may be the cause of abortion and stillbirth in cattle.

The aim of this study was to estimate the fertilization effectiveness of in heifers into relation with *U. diversum* carriage in the vulval vestibule. All 20 heifers in the study group were from the same dairy farm from Leningradskaya oblast. The samples were collected from the vulval vestibule by cotton swab. At the sample collection vulvar mucous appearance was estimated. The *U. diversum* carriage was detected by real-time PCR assay with diagnostic system "Ureaplasma diversum Amp" (St. Petersburg Institut Pasteur, Russia).

In the group of 20 heifers, 13 had symptoms or granular vulvovaginitis, including yellowish-gray pustules on the mucous. No other reproductive disease symptoms were detected in any heifer. The carriage of *U. diversum* was detected in 15 animals. The granular vulvovaginitis is commonly associated with *U. diversum* carriage in heifers and cows, but the symptoms of this disease are nonspecific and frequently may be associated with other diseases, for example with bovine rhinotracheitis, that is very widespread in cattle. No association was detected between granular vulvovaginitis symptoms and *U. diversum* carriage in study population.

The effectiveness of fertilization was estimated in all heifers. The average number of inseminations leads to fertilization in heifers without carriage was 1.2, but in infected heifers it was 1.9, and the difference between two groups was statistically significant ($t = 0.36$; $p < 0.002$). The fertilization failure was more frequent in heifers with *U. diversum* carriage. Twelve heifers from this group were fertilised at first insemination, two heifers in the same group were fertilized at second insemination and one heifer was inseminated six times before fertilization. Into the group without *U. diversum* carriage all but one of heifers were fertilized in the first insemination an one heifer in second insemination.

The loss in fertilization effectiveness leads to the economic burden in dairy farms due to costs of repeated inseminations and animal management. The appropriate diagnosis of *U. diversum* carriage in heifest may improve dairy farm productivity.

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ANTIBIOTIC-RESISTANT *KLEBSIELLA PNEUMONIAE* IN THE GUT MICROBIOTA OF HEALTHY INDIVIDUALS

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Klebsiella pneumoniae causes a wide range of infectious diseases including pneumonia, urinary tract infections, bacteremia and liver abscesses. Previously, it was believed that *K. pneumoniae* can cause serious infections primarily in people with decreased immunity, but the recent emergence and spread of hypervirulent strains has increased the number of people susceptible to these infections, including healthy ones. In addition, strains of *K. pneumoniae* are becoming more resistant to antibiotics, which creates special difficulties in treatment. Strains of the genus *Klebsiella* quite often colonize the mucous membrane of the distal gastrointestinal tract of children and adults, being part of the gut microbiota.

The aim of this study was to reveal frequency of occurrence of *K. pneumoniae* in intestinal microbiota of clinically healthy adults and children and to define antimicrobial susceptibility of isolated strains.

The microbiota content of 180 people aged from 1 month to 65 years was studied by quantative bacteriological method according to OST 91500.11.0004-2003 "Protocol of management of patients. Intestinal dysbacteriosis." *K. pneumoniae* strains were isolated in 25.0% (95% CI:19.2–31.8) of samples in quantaties exceeding 10^5 CFU/g. The susceptibility of these strains to 7 groups of antibiotics (penicillins combined with inhibitors, cephalosporins, quinolones, aminoglycosides, tetracycline, chloramphenicol, nitrofurans) was studied by disc diffusion method. 51.1% (95% CI:37.0–65.0) of the isolates were resistant to one or more antimicrobials. Multiple resistance (resistance to 3 or more classes) was found in 11.0% (95% CI:4.8–23.5) isolates. The highest resistance was observed to amoxicilline/clavulanic acid (31.1% of strains), the lowest — to amikacin (4.4% of strains). No strains resistant to carbapenems were found. Resistance to other antimicrobials ranged from 8.9% (chloramphenicol) to 22.2% (gentamicin).

The study showed that the intestinal microbiota of every fourth clinically healthy person contains strains of *K. pneumoniae*, half of which are resistant to one or more antimicrobials. At the same time, more than 10.0% of *K. pneumoniae* strains isolated from healthy people have multiple antibiotic resistance. Such strains can serve as a reservoir of determinants of resistance to other enterobacteria, including pathogens of acute intestinal infections.

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ANTIMICROBIAL RESISTANCE MECHANISMS IN BACTERIA STRAINS ISOLATED FROM FARM ANIMALS

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The aim of the work was to study the quinolone and β-lactame resistance mechanisms in *Salmonella* and opportunistic bacteria strains isolated from farm animals.

We determined the quinolones and β-lactams susceptibility and resistance mechanisms in 482 *Salmonella* and 144 strains of opportunistic bacteria (*E. coli, Klebsiella* spp.).

For 6 *Salmonella* strains (3 *S.* Enteritidis and 3 *S.* Infantis), resistant to fluoroquinolones the mutations in the QRDR region of *gyrA* gene were detected by amplification and sequencing of this DNA region (Kosyreva et al., 2012). The extended-spectrum β-lactamases (molecular classes A and C) were determined by PCR with specific primers (Dallenne et al., 2010). 132 strains of *Salmonella* were resistant to quinolones (27.4%), 41 of them (8.5%) had high level resistance to ciprofloxacin (MIC 6–32.0 mg/l). Sequencing of the *gyr*A of some resistant *Salmonella* isolates have been identified three types of single point mutations. In two *S.* Enteritidis the mutation was noted in 83 position (Serine replacement by Phenylalanine), in one strain — in 87 position (Asparagine replacement by Glycine). Three *S.* Infantis strains had the replacement of Asparagine by Tyrosine in 87 position.

9 strains of *Salmonella* (1.9%) were resistant to extended-spectrum cephalosporins. According the beta-lactamase inhibitor susceptibility tests, five of them were classified as ESBL-producers, 4 strains — as AmpC-producers. ESBL CTX-M was detected in strains of *S.* Haifa isolated from chicken samples and *S.* Derby isolated from the imported pork heart. AmpC cephalosporinase CMY was produced by two strains of *S.* Kentucky, isolated in 2006 and 2009 from imported poultry products, as well as two *S.* Dublin isolated in 2005 from the internal organs of a fallen calf and cow.

In opportunistic bacteria (*E. coli* and *Klebsiella* spp.) 22 strains were resistant to extended spectrum cephalosporins, 15 of them produced ESBL according the beta-lactamase inhibitor susceptibility tests. The class of detected β-lactamases was established in 11 strains. In *K. pneumoniae* and *K. ozenae* isolated from the milk of cows sick with mastitis ESBL CTX-M1 were detected. In *E. coli* isolated from calves suffering from diarrhea, was detected ESBL CTX-M1 and CTX-M9. So, our study has confirmed the circulation of *Salmonella* and other *Enterobacteriaceae* strains resistant to clinical significant antibiotics (fluoroquinolones and cefalosporines) in animal farms.

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DATA ANALYSIS OF MASS-SPECTRAL *KLEBSIELLA PNEUMONIAE* PROFILES TO PREDICT OF CARBAPENEM-RESISTANT STRAINS

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The increase of the number of enterobacteria with resistance to carbapenems has become a global problem. A rapid method for predicting resistance to carbapenems is needed, the results of which will be obtained even before the sensitivity to antibiotics is determined. In recent years, has been developing the trend, related to the increase in the MALDI-TOF mass spectrometry potential by bioinformatic approach to typing bacteria at the level of strains.

The study's aim was the data analysis of the *K. pneumoniae* mass spectra for protein biomarker discovery that make it possible to predict the detection of strains with OXA-48 and NDM-1 carbapenemase activity.

We used archived spectra obtained for the routine identification of isolates from hospital patients of St. Petersburg in 2015–2017. Digital data of 67 raw spectra, selected by identification results at the *K. pneumoniae* species level, were exported to the "BioNumerics" software. The created classifier was used to identify of new seven OXA-48 and eight NDM-1 strains pre-characterized by PCR, and 16 sensitive to meropenem *K. pneumoniae* strains. The biomarker peaks were designated by comparing their molecular weights with the data of plasmid proteins *K. pneumoniae* in the NCBI and UniProtKB bases with using the ExPASy portal.

The cluster analysis results of 67 spectra were used to create a model, that consist of six classes. The aggregate efficiency of the classifier was 89.6%. The spectra of group #4 had a marker peak m/z = 5996 Da, which was comparable in molecular weight to the protein pKF140-142 of plasmid pKF3-140. The marker peak m/z = 6096 of group #2 was designated as a plasmid protein according it coincidence on molecular weight with the protein UUU_02980 of plasmid pKPt2. Sixteen sensitive strains were mainly classified in group #2, but their spectra lacked a plasmid peak m/z = 6096. Eight NDM-1 strains were assigned to different groups, however their spectra showed either the peak of the plasmid protein m/z = 6096 or the peak m/z = 5936 which was also identified as a plasmid protein according to the protein of the outer membrane receptor protein of the plasmid pF77. All OXA-48 strains were assigned to group #4 and their spectra contained the peak of the plasmid protein m/z = 5996.

It has been suggested that the mass spectra of carbapenem-resistant *K. pneumoniae* strains may contain peaks attributed to the plasmid-encoded proteins. Such small plasmid proteins, which molecular weight don't correspond to the carbapenemases, even so, can appear as predict biomarkers of carbapenemase activity of strains.