9.12 doi: 10.15789/2220-7619-2018-4-9.12

DEVELOPMENT OF FUNCTIONAL NANOSTRUCTURES EFFECTIVE AGAINST BACTERIA BIOFILMS INCLUDING MULTIDRUG RESISTANT BACTERIA

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The emergence and distribution of antibiotic resistant strains of bacteria is one of the most world's pressing public health problems. Multidrug resistant (MDR) bacteria exhibit resistances to most antibiotics and, sometimes, to nearly all commercially available antibiotics. Besides, the persistence of bacteria in the body mainly in the state of biofilm reduces significantly the effectiveness of antibacterial therapy. Even antibiotic-sensitive strains in the state of the biofilm are weakly responsive towards antibiotics. There is an urgent need for development of new antibiotic treatment strategies against MDR bacteria and bacteria biofilms. The most potentially successful strategy may be the transition from classical therapy to the use of high-tech tools based on nanomaterials.

The purpose of this study was the development of new antimicrobial agents based on nanostructured materials of organic and inorganic origin. Antibacterial properties of nanostructured classical antibiotics (tetracycline), metal particles and their oxides, magnetically controlled nanocomposites with encapsulated antibiotic (ciprofloxacin) were investigated.

The preliminary results demonstrate the high antimicrobial activity of the developed materials. Nanostructuring of tetracycline increased its effectiveness up to 40% against a resistant strain, compared with the original antibiotic form. Particles of metals and their oxides showed excellent antimicrobial properties, including against antibiotic-resistant strains, but many of them can cause some cytotoxic effect in the macroorganism. Magnetically controlled nanocomposites with encapsulated ciprofloxacin demonstrated an increase in efficiency of up to 76% compared to the initial form of the antibiotic due to magnetically controlled effects of mechanical disintegration of the biofilm, accumulation and release of the composite inside the biofilm. Also, synergism in antibacterial action may be due to local alkalization due to recrystallization of calcium carbonate.

Thus, the results of this study can create a scientific basis for the development of new antimicrobial agents based on nanostructured materials effective against biofilms and antibiotic-resistant strains of bacteria.

This work was supported by the Ministry of Education and Science of the Russian Federation (Project 4.8955.2017/8.9).

9.13 doi: 10.15789/2220-7619-2018-4-9.13

KLEBSIELLA PNEUMONIAE AND ITS GENES OF RESISTANCE TO BETA-LACTAMAMS IN PSYCHIATRIC HOSPITAL

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The aim of the study was to characterise of phenotype and genotype of antibiotic resistance in *Klebsiella pneumoniae*, isolated in psychiatric hospital.

215 strains of *K. pneumoniae*, isolated from sputum, urine, wounds and blood of the patients in 2016 were studied. Bacteria were identified using classical methods, antibiotic resistance was studied according to MUK 2004, Clinical guidelines for antimicrobial sensitivity determination, 2015. Beta-lactamases gene detection was performed by PCR.

Klebsiella showed high resistance levels to inhibitor-protected penicillins (86.5%) and cephalosporins (77.7%). Resistant to fluorochinolones were 51.6% strains, to carbapenems (meropenem) — 32.6% of isolated strains. The lowest resistance level was observed in amikacin and fosfomycin — 17.2% and 4.6% resistant strains respectively. Multidrug resistant were 59.3% of isolated *K. pneumoniae* strains. Almost a quarter — 24.7%, of strains showed associated resistance to inhibitor-protected penicillins, cephalosporins, fluoroxhinolones and carbapenems. Extreme antibiotic resistance was observed in 9.8% isolates — they were sensitive to colistin. Detection of beta-lactamases genes was performed in 30 cultures, bla_{CTX} was found in 80.0% of strains, bla_{TEM} — in 70.0%, bla_{OXA-48} and bla_{NDM-1} were found in 6.7% and 86.7% of strains respectively; bla_{OXA-48} gene was combined with bla_{NDM-1} in 6.7% isolates. The most frequently encountered beta lactamases were combination of bla_{CTX}, bla_{TEM} and bla_{NDM-1} (70.0%). Other genes combinations were revealed less frequently: bla_{CTX}, bla_{TEM} and bla_{NDM-1} were present in 6.7% strains, bla_{CTX} and bla_{NDM-1} — in 3.3%. Bla_{NDM-1} only was detected in 6.7% *K. pneumoniae*,

Resistant to antibiotics strains of *K. pneumoniae* prevailed in psychiatric hospital, third of isolates were resistant to carbapenems. The most common combination of resistance determinants for carbapenem-resistant strains was bla_{CTX}, bla_{TEM} and bla_{NDM-1} — 70%. Spread of carbapenemases, mostly NDM-1 producing *K. pneumoniae* strains, is a dangerous sign of the significant decrease in carbapenems efficacy towards infections, caused by *K. pneumoniae* and confirm the necessity of antimicrobial resistance monitoring in hospitals strains.

9.14 doi: 10.15789/2220-7619-2018-4-9.14

RESISTANCE TO ANTIBIOTICS OF DIARRHEAGENIC *ESCHERICHIA COLI* IN PSYCHIATRIC HOSPITAL

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The aim of the study was estimation of antibiotic resistance in diarrheagenic *Escherichia coli*, isolated in psychiatric hospital in St. Petersburg.

Study of susceptibility to antibiotics in 123 strains of *E. coli* was carried out according to guidelines MUK 4.2.1890-04, 2004. Cultures were isolated from feces of patients of a psychiatric hospital in 2016–2017.

Diarrheagenic *E. coli* in psychiatric hospital were represented by enteroinvasive (48.0%) and enterotoxigenic *E. coli* (47.2%) mainly. Ratio of enterohemorrhagic *E. coli* (EHEC) was small (4.9%). Enteroinvasive *E. coli* (EIEC) included representatives of 3 serogroups, with prevalence (34.1%) of O144. Other serogroups were rare (8.1% for O151 and 5.7% for O124 strains). Enterotoxigenic *E. coli* (ETEC) included representatives of 3 serogroups, most common were O6 (26.1%) and O25 (20.3%). Only one strain of O85 serogroup (0.8%) was isolated in 2017. EHEC was presented with one serogroup O1 (4.9%). Number of strains of diarrheagenic *E. coli* increased two-fold in 2017 compared with 2016. The proportion of the EIEC O144 increased eight-fold. There was more than two-fold increase of ETEC O25. Proportion of *E. coli* belonging to other serogroups changed slightly with the exception of EHEC O1, the number of strains decreased from 5 in 2016 to 1

in 2017. Study of susceptibility of *E. coli* showed that resistant to antibiotics strains prevailed (61.8%) and the ratio of such strains increased almost two-fold in 2017 (72.1%) from 2016 (43.2%). Most isolates were resistant to ampicillin and inhibitor-protected penicillins such as amoxicillin/clavulanate (61.0%). Proportion of such strains increased almost two-fold in 2017 (72.2%) from 2016 (40.9%). Resistant to fluoroquinolones, cephalosporins were 3.2% and 2.4% strains, more than 20 times less. All of them were isolated in 2016. Isolates resistant to two preparations at the same time were most frequent (58.5%). The proportion of multidrug resistant cultures was low (2.4%). There were only 3 isolates resistant to ampicillin, inhibitor-protected penicillins, fluoroquinolones and cephalosporins.

Number of strains of diarrheagenic *E. coli* increased two-fold in 2017 compared with 2016, most of them were EIEC O144 and ETEC O25. Antibiotic-resistant cultures prevailed. Multidrug resistant strains were rare. All strains were susceptible to carbapenems, most of them — to fluoroquinolones and cephalosporins.

9.15 doi: 10.15789/2220-7619-2018-4-9.15

MOLECULAR ANALYSIS OF PATHOGENS OF PARTICULARLY DANGEROUS BACTERIAL INFECTIONS: FROM THEORY TO PRACTICE

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The modern period of development of medical and biological science is characterized by significant successes in the field of structural analysis of microorganisms and wide technological possibilities of obtaining living objects with given properties. A new scientific trend has emerged — synthetic biology. One of the urgent goals is the application of molecular methods in the practice of epidemiological analysis, to determine the source of infection and the pathways of the spread of the microbial pathogen, to assess its virulence and other properties.

At the present time, considerable material has been accumulated on the genetics of pathogens of anthrax, plague, cholera, brucellosis and other extremely dangerous microorganisms. The algorithms of PCR analysis have been developed in determining the epidemiological significance of isolate strains and their taxonomic features. There is experience of genotyping using MLVA, MLST, SNP and other methods, as well as the analysis of the complete genomic sequence (WGS).

Taking into account the levels of strain analysis (diagnosis of infection or epidemiological analysis), the following current research areas can be identified:

– Detection and identification: application of nucleic acid amplification methods for the differentiation of living and dead cells; introduction of multiplex (multifactor) PCR analysis technologies; creation (completion) of databases of mass spectra of microorganisms; introduction of methods of direct mass-spectrometric analysis of clinical material.

– Molecular typing: the creation of sequential (optimal) genotyping algorithms for each species; application of protein profiling methods for typing pathogens.

– Application of information systems, epidemiological analysis: creation of own databases of full-genomic sequencing; genomic profiling of pathogens in specific areas; creation of complex software products using the data of geographical information systems and predictive modeling.

As a result, an algorithm for bioinformational analysis should be developed for the epidemiological investigation of outbreaks (cases) of infectious diseases, including those caused by new (atypical) genetic variants of pathogens of especially dangerous infections.

9.16 doi: 10.15789/2220-7619-2018-4-9.16

DIFFERENTIATION OF *KLEBSIELLA* spp. STRAINS FOR SENSITIVITY TO ANTIBIOTICS USING MASS SPECTROMETRY ANALYSIS MALDI-TOF

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Klebsiella spp. strains are frequent causative agents of health care-related infections. Those strains especially dangerous if they circulate in hospitals. They are usually antibiotic-resistant. Therefore, information about the sensitivity of the isolated strain is necessary in the shortest time for proper etiological treatment.

The aim of our study was to assess the possibility of using mass spectrometry analysis (MALDI-TOF) for the rapid prediction of a selected *Klebsiella* spp. strain resistance.

The study used 195 strains of *Klebsiella* spp. isolated in various medical centers of St. Petersburg. All strains were identified by MALDI-TOF. Antibiotic sensitivity was studied by the disco-diffusion method in accordance with the recommendations of EUCAST 8.0. We used 19 antibiotics from 5 classes for testing strains: aminoglycosides, beta-lactams, beta-lactams of extended spectrum, quinolones and carbapenems.

A hierarchical clustering of spectra was made using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to determine the relationship between clusters. We used Pearson correlation coefficient between variable values of peak intensity in spectral profiles as a measure of the distance between individual mass spectra. We identified 2 significant difference in the spectrum of the cluster. One cluster included spectra of strains resistant to all studied classes of antibiotics, second cluster — strains sensitive to them (Distance Level — 0.65). It was also found that all strains included in the clusters, which differ from others by Distance Level more than 0.2, have the same profile of antibiotic resistance.

As a result of this work, we have formed profiles of phenotypic resistance of *Klebsiella* spp. strains to 19 antibiotics and 5 classes. The prospect of using the results of the study is a significant reduction in the study of biological material from the patient. Thus, simultaneously with the identification of *Klebsiella* spp. strains by MALDI-TOF it is possible to predict the sensitivity of the isolated strain to different classes of antibiotics or even to one of them. This will allow timely recognition of resistant strains of *Klebsiella* spp and prescribe adequate etiological therapy, which will significantly improve the quality of treatment of patients and will prevent the spread of resistant strains of bacteria in the medical establishments.

9.17 doi: 10.15789/2220-7619-2018-4-9.17

PATHOGENIC POTENTIAL OF COMMENSAL *ESCHERICHIA COLI* ISOLATED FROM ADULTS IN SAINT PETERSBURG

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Escherichia coli is one constitute a component of the natural microbiota of warm-blooded animals including humans. At the same time commensal *E.coli* is a dynamic population and in some cases is capable to cause ex-