MOLECULAR-GENETIC TYPING AND STUDYING OF ANTIBIOTIC SENSITIVITY OF THE P.AERUGINOSA NOSOCOMIAL STRAINS IN MULTI-TYPE CLINIC

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The article presents the results of molecular genetic typing and studying of resistance to antibiotics of *Pseudomonas aeruginosa* nosocomial strains, isolated from the clinical samples of the patients who were hospitalized in different units of Republican Hospital №2 – Center of Emergency Medical Care in period of 2010-2011. Clonal spread of multidrug-resistant (XDR) metal-beta-lactamase-producing *P.aeruginosa* strains ST235 (VIM-2) were revealed.

Key words: Pseudomonas aeruginosa, nosocomial infections, antimicrobial resistance, sensitivity to antibiotics, metal-ß-lactamase

Abstract. Nosocomial infections are a serious problem in modern medicine and have significant impact on prognosis and outcome of disease. One of the most serious pathogens of nosocomial infections complicating course of many chronic inflammatory diseases is Pseudomonas aeruginosa (P.aeruginosa). The special features of this organism are rapid formation and high level of resistance to many wide range antibiotics, which is usually prescribed for empirical treatment of nosocomial infections [2].

P.aeruginosa characterized by different mechanisms of resistance – reducing of cell wall permeability to antibiotics (lower OprD protein expression), active excretion of antibiotic from the cells (activation of efflux systems), production of individual serine β-lactamases with Carbapenem activity. However, most clinical and epidemiological importance production of metal-b-lactamase (MβL) has. Metal-b-lactamase IMP and VIM hydrolyzes all b-lactams in practice, including Carbapenems, except Aztreonam.

Therefore, effective treatment of infections caused by P.aeruginosa remains a complex clinical problem and requires adequate microbiological control and compulsory studying of their sensitivity in vitro [3].

Objective. The object of our investigation was to determine the antibiotic resistance levels,

prevalence of metal-b-lactamase and genetic typing of P.aeruginosa strains, isolated from multi-type clinic patients.

Materials and methods.

During two years (2010-2011), the 662 isolates of P.aeruginosa, isolated from clinical materials of patients from different units of Republican Hospital №2 – Center of Emergency Medical Care (unit of urgent surgery, unit of purulent surgery, resuscitation and intensive care unit, burns unit, emergency department, unit of patients with stroke, intensive care unit for patients with stroke, neurosurgery unit), were studied.

Identification and re-identification of the strains by the conventional methods according to the documents regulating work of bacteriological laboratories were performed. Also we used a biochemical microtest MICROLATEST, API system test (bioMerieux, France).

Study was conducted within project "National program for monitoring spread of the Gram-negative microorganism strains producing metal-ß-lactamase in Russia (METAL)" together with Scientific Research Institute of Antimicrobial Chemotherapy of Smolensk State Medical Academy of Russian Ministry of Health, Interregional Association of Clinical Microbiology and Antimicrobial Chemotherapy, Scientific Methodological Center of Antimicrobial Resistance.

Sensitivity to the 15 antimicrobial drugs by disc-diffusion method on Mueller-Hinton medium according to methodical instructions 4.2.1890-04 was determined. Quality control was performed using the control strains of P.aeruginosa ATCC 27853, E. coli ATCC 25922. Interpretation of results was carried out in accordance with guidelines and criteria of CLSI / NCCLS (2010-2011).
Phenotypic screening of products of metal-beta-lactamase (MβL) by double disc method with EDTA for the all Carbapenem resistant strains was performed [4].

To detect genes MβL VIM and IMP types we used multiplex polymerase chain reaction in real time (28 multiresistant clinical isolates of P.aeruginosa).

Identification of the amplification fragments of the bla_{VIM} and bla_{IMP} genes was carried out by determining their melting point (~ 80°C for bla_{IMP} and ~ 85°C for bla_{VIM}) in the presence of intercalating fluorescent dye SYBR Green I. In addition, the melting curves of the experimental samples were compared with the melting curves of the positive control strains.

To evaluate structure of the integrons, carrying the genes MßL, RFLP (restriction fragment length polymorphism) method was used. The variable sections of the class I integrons was amplified using the primers to 5’ (intI1) and 3’ (qacEΔ1 or tniC/Tn5090) conservative sequences of

the integrons in pairs with the internal primers to the bla_{VIM} genes and was subjected to restriction by the endonuclease TaqI. Obtained restriction profiles of the PCR fragments were compared with corresponding profiles of the known MßL-encoding integrons, used as controls.

Epidemiological typing of the Carbapenem resistant isolates of P.aeruginosa, using multiple-locus variable number tandem repeat analysis (MLVA), was performed. Number of tandem repeats in the six VNTR-loci (VNTR - Variable Number Tandem Repeat, tandem repeats with variable number of the links) was estimated. Amplification of the six VNTR-loci was performed using multiplex PCR (2 separate reactions for each isolate). Analysis of the size of amplification products of the six VNTR-loci was carried out by capillary electrophoresis with fluorescence detection (a fragment analysis) on an automatic sequencer ABI-310 Genetic Analyzer (Applied Biosystems). Cluster analysis of MLVA profiles was performed by software package Bionumerics v.6.6 (Applied Maths) using categorical values of the lengths of the VNTR-loci and algorithm for the construction dendrograms of minimum distance (Minimum Spanning Tree) [4].

Input, processing and statistical analysis was performed using computer program Microsoft Excel (version 7.0. for Windows 2000) and software WHONET 5.6.

Results and discussion.

Greatest number of the P.aeruginosa strains – 42,8% (283 isolates), was revealed in the patients treated at burns unit. Share of the identified P.aeruginosa strains in other hospital departments was as follows: resuscitation and intensive care unit – 24,4% (162), unit of purulent surgery – 15,5% (102), neurosurgery unit – 8,1% (54), unit of urgent surgery – 2,9% (19), intensive care unit for patients with stroke – 2,4% (16), unit of patients with stroke – 2,2% (15) and emergency department – 1,7% (11).

The clinical isolates of P.aeruginosa (n=662) with high frequency were isolated from the wound discharge – 428 (64,6%), than in descending order from the tracheal aspirate – 91 (13,8%), bronchial wash-water – 81 (12,2%), sputum – 42 (6,4%), pleural fluid – 20 (3%) (Fig. 1).

Using the "Double disk with EDTA" method in the 662 strains, MßL production was detected in the 223 (33,6%) Carbapenem resistant strains of P.aeruginosa from the 7 different units of Republican Hospital №2 – Center of Emergency Medical Care.

At the next stage of work we have conducted molecular genetic study of the 28 Carbapenem resistant strains of P.aeruginosa. In the all 28 isolates the presence of VIM-type MßL was confirmed by PCR analysis.

Method of RFLP (restriction fragment length polymorphism) established identity of structure of the integrons carrying the gene MßL in these isolates and in the VIM-2-encoding

integron with the set of genetic cassettes: aacA7-bla$_{VIM-2}$-dhfrB5-aacC-A5 (GenBank Acc. No. DQ52233) (Fig. 2), previously described in the strains of P.aeruginosa from the U.S. [6], Russia [7] and Norway [8].

Typing of P.aeruginosa by multiple tandem repeats analysis (MLVA) revealed that the all 28 MßL-positive strains of P.aeruginosa are related and belong to single clonal complex (CC235) (Fig. 3). This common sequence-type 235 (ST235) is epidemic now. It was detected in hospitals of 27 cities of Russia, Belarus and Kazakhstan (project "National program for monitoring spread of the Gram-negative microorganism strains producing metal-ß-lactamase in Russia (METAL)"

Determination of sensibility of the MßL-producing P.aeruginosa isolates showed that the isolates are characterized by high levels of resistance (100%) to the all tested antimicrobial drugs: Piperacillin, Piperacillin-Tazobactam, Ceftazidime, Cefepime, Cefoperazone-Sulbactam, Aztreonam, Imipenem, Meropenem, Doripenem, Gentamicin, Netilmicin, Amikacin, Ciprofloxacin, Levofloxacin, Phosphomycin. The sensitivity of isolates identified only to Colistin and Polymyxin B.

Conclusions:

1. In multi-type clinic “Republican Hospital №2 – Center of Emergency Medical Care” clonal spread of the super-resistant (XDR) strains of P.aeruginosa ST235 (VIM-2) were detected.
2. Spread of antibiotic poly-resistance among the isolates of P.aeruginosa in clinical departments of “Republican Hospital №2 – Center of Emergency Medical Care” is conditioned by one of most common mechanism of antibiotic resistance associated with metal-ß-lactamase production.
3. The MßL-producing isolates of P.aeruginosa were detected in the all clinical materials and practically in all units of multi-type clinic of the Republic of Sakha (Yakutia).
4. Revealing the MßL-producing strains and the dangerous epidemic clones of P.aeruginosa requires the development of infection control measures aimed at early detection and limitation of circulation the MßL-producing isolates of P.aeruginosa in hospital departments and other medical institutions, introduction of permanent monitoring of antibiotic resistance, epidemiological tagging of the Carbapenem resistant isolates.