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RESEARCH ARTICLE

THE SENSITIVITY TO ANTIBIOTICS OF PSEUDOMONAS AERUGINOSA STRAINS, ISOLATED IN NEWBORNS

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ABSTRACT

Introduction: The rationale for conducting research is high frequency of morbidity and mortality among newborn infants due to the infection caused by Pseudomonas aeruginosa.
Objective: There was the study of antibiogramm of 117 strains of Pseudomonas aeruginosa. Pathogens were isolated from the different loci (the respiratory tract, mucous eye, umbilical wound, ear, blood, urine) among 117 newborns.
Methods: The determination of Pseudomonas aeruginosa's sensitivity to antibiotics by standard discs method.
Results: The 10 strains were nosocomial and referred to the three genetic types (3 strains - I and II type, 4 strains - III type). The 107 clinical strains were individual ("wild-type"). All the strains were sensitive to Piperacillin, Piperacillin + Tasobactam, Colistin and resistant to Carbenicillin, Doxycycline, Cefaclor, Chloramphenicol. The sensitivity to the rest of the 15 antibiotics was variable.
Conclusions: The nosocomial and individual strains Pseudomonas aeruginosa are polyresistant to antibiotics. The systematic implementation of microbiological monitoring should be (2-4 times per year) in the stationary and also the antibiotics' rotation has to be applied.

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INTRODUCTION

The modern neonatology made considerable progress in nursing and treating of premature newborn infants (American Academy of Pediatrics, 2015; Zubkov *et al*., 2014). However, the incidence of nosocomial infections in these patients still remains high (Svitich *et al*., 2016), and the mortality rate among the diseased reaches 38% (Duenas *et al*., 2011). It is known that the causative agents of nosocomial infection are able to change their biological properties, in particular, sensitivity to antibiotics. It is connected with the widespread use of antimicrobials in medicine, veterinary medicine, food industry and also in agriculture, and creates difficulties in the selection of the adequate antibiotic treatment (Chetverik *et al*., 2012; Duenas *et al*., 2011; Svitich *et al*., 2016; Zubkov *et al*., 2014). In this connection, it is necessary to investigate the prevalence of antibiotic-resistant bacterial strains, to determine the spectrum of the medicines used and, if it is necessary, to develop new antibacterials. During the last 10 years, the infection is still relevant, including infection in preterm newborn infants which is caused by Pseudomonas aeruginosa (*P.aeruginosa*) (American Academy of Pediatrics, 2015; Cernada *et al*., 2013; Chetverik *et al*., 2012; Chubenko *et al*., 2012; L'Heriteau *et al*., 2012; Lubarovskaya *et al*., 2013). These diseases are compound from 13 (L'Heriteau *et al*., 2012) to 39% (Lubarovskaya *et al*., 2013) of all cases of the neonatal hospital infection. The epidemiological importance of this pathogen is largely owing to its biological properties, specifically, the formation and proliferation of new hospital strains, polyresistance to antibiotics, the ability to remain for a long time on moist surfaces of the objects in environment, including medical equipment parts (American Academy of Pediatrics, 2015; L'Heriteau *et al*., 2012; Maltezou *et al*., 2013; Methods of control, 2004; Zubkov *et al*., 2014). In our opinion, it is important to perform a detailed analysis of *P.aeruginosa* strains sensitivity to antibiotic in a large group of the diseased patients. This will determine the optimal treatment of hospital infections in newborn infants and to fight successfully the nosocomial infection in the intensive care unit of the hospital.

Objective

To investigate Pseudomonas aeruginosa sensitivity to antibiotic strains which are obtained in newborns with the nosocomial infection.

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MATERIALS AND METHODS

We observed 117 newborns with the nosocomial infection caused by *P. aeruginosa*. Among them there were 60 girls and 57 boys. 80 babies were premature. The body weight at birth was varied from 680 to 2580 g (2001.5 ± 182.3 g) and the gestational age was from 26 to 37 weeks (32.3 ± 0.68 weeks). 37 babies were mature with the birth weight from 2880 to 4100 g (3922±153.3 g) and the gestational age of 38 to 41 weeks (40.2±1.04 weeks). Hospital infection caused by *P.aeruginosa* was presented to "ventilator - associated pneumonia" (65 infants), tracheobronchitis (12), rhinitis (10), omphalitis (18), conjunctivitis (5), otitis media (2), urinary tract infection (4), sepsis (1). The microbiological examination of tracheobronchial aspirates (TBA) and oral swabs from the back of the throat, blood, urine, discharge from the umbilical wound, ear and eyes. This examination was performed by standard quantitative method for all infants for a wide range of nutrient media for the isolation of aerobian and facultative microorganisms (Menshikov, 2003).

All bacterial cultures were identified by commercial test systems API 20 NE «BioMerieux» (France), E | NE "Crystal" (USA), NEFERMtest "Lachema" (Czech Republic) in accordance with the manufacturer's instructions. The number of microorganisms was expressed in the following units: 1) The number of colony forming units in 1 standard tampon (CFU / t) or biofluid in 1 ml (CFU / ml) in microbial contamination up to 1000 cells. 2) The decimal logarithm (lg) in microbial load 1000 and more microbial cell in 1 tampon or 1 ml of biofluid. Etiologically significant number of microbial cells believed lg4 and higher CFU / ml for TBA, lg 6 and higher CFU / t for the smear with mucous posterior pharyngeal (Menshikov, 2003). Sensitivity determination of isolated strains *P.aeruginosa* to antibiotic conducted disk-diffusion method on agar Mueller - Hinton (Mueller Hinton Agar) using standard commercial drive test systems NITSF (Russia) and test systems ATV pse 5 (BioMerieux) (Methods of control, 2004). The study was conducted in Moscow (Russia) in the City Clinical Hospital №13 from 2011 to 2015.

RESULTS

117 strains of *P.aeruginosa* were isolated from the examined infants. All selected strains of *P. aeruginosa* were with hemolytic properties, elastase and phospholipase activities. There were isolated *P.aeruginosa* strains in all newborns, including 77 strains from the respiratory tract, 18 strains from the umbilical wound, 4 urine, 2 strains from the ear, and 5 from the eye and 1 from the blood. *P.aeruginosa* was isolated at the same time from the different examined locus in 12 babies. These strains were identical in their biological properties in each baby. 10 strains of *P. aeruginosa* were hospital strains. They were isolated from the respiratory tract in newborn with tracheobronchitis. Three groups of hospital strains were identified based on the study of antibiogramm and biochemical properties of *P.aeruginosa* strains. Group I of *P.aeruginosa* strains (3 strains) were sensative to Piperacillin, Piperacillin + Tasobactam, Amicacin, Gentamicin, Ofloxacin, Ciprofloxacin and Colistin. The strains from group I were resistant to other antibiotics under the study. *P.aeruginosa* strains (3 strains) of group II were sensitive to Piperacillin, Piperacillin + Tasobactam, Amicacin, Gentamicin, Ciprofloxacin and Colistin, and had intermediate sensitivity to Imipenem / Cilastatin, Meropenem, Ofloxacin. *P.aeruginosa* strains (4 strains) of group III was sensitive to Azlocillin, Piperacillin, Piperacillin+Tasobactam, Amicacin, Ceftazidime, Ceftriaxone, Cefoperazone, Ciprofloxacin, Ofloxacin, Tobramicin, Nitroxoline and Colistin, and it also had intermediate sensitivity to Carbenicillin and Cefotaxime. Similar hospital strains of *P. aeruginosa* were detected by us in newborns with VAP in the previous study (Kushnareva *et al*., 2017). All three types of hospital strains of *P. aeruginosa* were sensitive to amikacin, colistin, piperacillin, piperacillin-tazobactam and ciprofloxacin and resistant to doxycycline, kanamycin, chloramphenicol and rifampin. *P. aeruginosa* strain of type I was resistant to 16 studied antibiotics, *P. aeruginosa* strain of type II - to 12 antibiotics and *P. aeruginosa* strain of type III - to 7 antibiotics. As was noted earlier, Finding of one stationary 3 variants of hospital strains *P. aeruginosa* among patients may

Table. The sensitivity of clinical strains to antibiotics *P.aeruginosa* (n=107)

	Antibiotics	S		R		SR	
		n	%	n	%	n	%
1	Azlocillin	51	48	56	52	0	0
2	Meropenem	57	53	28	26	22	21
3	Imipenem/cilastatin	56	52	28	26	23	22
4	Piperacillin	107	100	0	0	0	0
5	Piperacillin + Tasobactam	107	100	0	0	0	0
6	Carbenicillin	0	0	107	100	0	0
7	Cefotaxime	16	15	71	66	20	19
8	Ceftazidime	51	48	49	46	7	7
9	Ceftriaxone	22	21	68	64	17	16
10	Cefoperazone	71	66	36	34	0	0
11	Cefaclor	0	0	107	100	0	0
12	Doxycycline	0	0	107	100	0	0
13	Amicacin	67	63	35	33	5	5
14	Gentamicin	23	21	80	75	4	4
15	Kanamycin	5	5	10	94	1	1
16	Ofloxacin	64	60	9	8	34	32
17	Ciprofloxacin	34	32	64	60	9	8
18	Chloramphenicol	0	0	107	100	0	0
19	Rifampicin	7	7	98	92	2	2
20	Tobramicin	31	29	70	65	6	6
21	Nitroxoline	9	8	71	66	27	25
22	Colistin	107	100	0	0	0	0

S – sensor strain, R – sustained strain, SR – strain with an intermediate sensitivity.

point to their possible formation in the health facility or their getting from other clinics while transferring the newborn (Baranov and Kornachev, 2015; Svitich *et al*., 2016). The study of sensitivity to antibiotics of other 107 clinical strains of *P. aeruginosa* are shown in Table. All 107 clinical *P.aeruginosa* strains ("wild-type strains") had individual antibiogram and saved sensitivity to piperacilinu, piperacillin / tazobactam and colistin.More than half of the tested strains were sensitive to Meropenem, Imipenem/cilastatin, Azlocillin, Cefoperazone, Ofloxacin, Amicacin. Thus all strains were multiresistant to a different spectrum of antibiotics. Noteworthy is the stability of all "wild-type" of clinical strains to Doxycycline, Carbenicillin, Cefaclor, Chloramphenicol and the most strains to Cefotaxime, Ceftriaxone, Ciprofloxacin, Gentamicin, Kanamycin, Tobramicin, Nitroxoline, Rifampicin. This fact indicates on the widespread polyresistant to antibiotics *Ps.aeruginosa* strains among the population and probably it is connected with the numerous and long-term use of these antibiotics in the region.

DISCUSSION

The common tendency was observed in the comparison of our study's results with the other authors' results (American Academy of Pediatrics, 2015; Baranov and Kornachev, 2015; Cernada *et al*., 2013; Chetverik *et al*., 2012; Duenas *et al*., 2011; Maltezou *et al*., 2013). First of all, there is a great variability antibiogram, in the second place, *Ps.aeruginosa* polyresistance is pathogenic to a large number of broad-spectrum antibiotics, and, in the third place, many strains *Ps.aeruginosa* (including nosocosmial) remain sensitive to Piperacillin, Piperacillin + Tasobactam and Colistin. The microbiological examination of *Ps.aeruginosa* strains (including their genotype test and antibiogram determination) allows to reveal the hospital strains, their sources in the environment and among medical staff and then to conduct the necessary sanitary measures. Moreover, such studies of the biological properties of pathogens of the nosocomial infection are the basis for the schemes' development of the empirical antibiotic therapy in the stationary. According to several authors, the conduction of microbiological monitoring at the clinic once in 3 - 6 months allows to improve significantly the effectiveness of the newborn's treatment (American Academy of Pediatrics, 2015; Chubenko *et al*., 2012; L'Heriteau *et al*., 2012; Zubkov *et al*., 2014).

Conclusion

Thus, the study of the biological properties of strains of *Ps.aeruginosa* was discovered three groups of hospital strains and clinical individual strains ("wild-type strains") of *Ps.aeruginosa*. The polyresistance to the most studied antibiotics was characterized for hospital strains that made it difficult to conduct the empirical treatment. The sensitivity to antibiotics of individual *Ps.aeruginosa* clinical strains (which did not belong to the selected group of the hospital strains) was very variable. However, these strains were polyresistant to 8-14 antibiotics. The high prevalence of *Ps.aeruginosa* strains polyresistant to antibiotics dictates the necessity of rotation of the applied antibacterial medicines and systematic microbiological monitoring in the stationary (2-4 times per year).

Conflict of Interest: The authors have no conflict of interest.

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