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Research Article

Ventilator-Associated Pneumonia Caused by *Pseudomonas Aeruginosa* in Preterm Newborn Infants

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Abstract

Study's Background and Aim: High incidence rate of VAP including those caused by *Pseudomonas aeruginosa* among preterm infants.

Objective: To investigate the clinical features of ventilator - associated pneumonia caused by Pseudomonas aeruginosa in premature newborns and properties of strains - pathogens.

Materials and Methods: In total 35 premature infants with VAP, caused by *Pseudomonas aeruginosa* were examined. Isolation and identification of microorganisms was carried out by conventional methods. Genotyping of strains of *P. aeruginosa* was performed by polymerase chain reaction and electrophoresis. The sensitivity of strains of pathogens to antibiotics was determined by the disk - diffusion method using standard drives.

Results: Most premature infants VAP was preceded in severe form of toxicosis, trophic disorders, severe respiratory failure, bilateral, often common pulmonary and bronchial tubes. In 28 newborns had complications in the form of atelectasis, pneumothorax, hemorrhagic syndrome, bronchopulmonary dysplasia. The mortality rate was 23%. 25 clinical strains and 3 different groups of hospital strains of *P. aeruginosa* were marked. All strains had hemolytic and elastase activity. They were multi-resistant to 12 - 19 antibiotics, but maintained sensitivity to piperacillin and colistin.

Conclusion: Usually VAP caused by *P. aeruginosa* has severe course with high mortality. Pathogens are polyresistant to antibiotics and they have a wide variety antibiograms.

Keywords: *Preterm Newborn Infants; Ventilator-Associated Pneumonia; Diagnosis; Clinical Course; Pseudomonas aeruginosa*

Introduction

The modern neonatology made considerable progress in nursing and treating of premature newborn infants. However, the problem of nosocomial ventilator-associated pneumonia (VAP) in this category of patients is still relevant. This disease is accounted for 20% of nosocomial infections [1]. Although many scientific studies are devoted to the problem of VAP in premature newborn infants [1-5], however, there are few works concerned with the study of the clinical course of the disease [5,7,8].

During the last 10 years, the infection is still relevant, including VAP in preterm newborn infants which is caused by *Pseudomonas aeruginosa* [1,5-7]. The epidemiological importance of this pathogen is largely owing to its biological properties, specifically, the formation and proliferation of new hospital strains, poly resistance to antibiotics, the ability to remain for a long time on moist surfaces of the objects in environment, including medical equipment parts [2,9,10]. In our opinion, it is important to make a detailed analysis of the clinical course of VAP, caused by *Pseudomonas aeruginosa (P. aeruginosa)* in a large group of patients and study of the biological properties of pathogens. This will allow to determine optimal treatment VAP in preterm infants and successfully fight nosocomial infections in Intensive care unit. It will allow to determine an optimal treatment VAP in preterm newborn infants and successfully fight with nosocomial infections in resuscitation and intensive care unit.

Objective

To research the clinical features of ventilator-associated pneumonia caused by *Pseudomonas aeruginosa* in premature newborn infants and properties of strains – pathogens.

Materials and Methods

We observed 35 preterm newborn infants with VAP caused by *P. aeruginosa* as monoinfection in 29 children and mixed infection in 6 infants. Body weight at birth varied from 680 to 2580 g (2001.5 ± 182.3 g) and gestational age was from 26 to 37 weeks (32.3 ± 0.68 weeks). Among them there were 11 girls and 24 boys.

The microbiological examination of tracheobronchial aspirates (TBA) and oral swabs from the back of the throat was examined in all infants. This examination was performed by standard quantitative method for all infants for a wide range of nutrient media for the isolation of aerobic and facultative organisms [11]. The study was conducted at onset of VAP and days 2 - 3 times in the course of the disease after 7 - 14. 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 / m) 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 [11].

Sensitivity determination of isolated strains bacteria (*P. aeruginosa* and bacteria - Assiociants) 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) [12].

3) Genotyping strains of *P. aeruginosa* was conducted by PCR and agarose gel electrophoresis using different sizes of arbitrary primers to detect hospital strains [13].

Results

Maternal Health: All the children were born prematurely in women with a history of physical and obstetric-gynecologic anamnesis. There were 21 primipara mother, and 14 multipara mothers. Four women had multiple pregnancies. Chronic somatic diseases such as (asthma, bronchitis, pyelonephritis, gastritis, and others) were observed in 83% of women, and chronic obstetric-gynecologic diseases (habitual miscarriage, infertility, adnexitis, cervical erosion, colpitis, uterine fibroids) occurred in 69%. Pregnancy complications as toxicosis in the 1st half and gestosis were observed in 13% and 66% of women, respectively, threatening miscarriage in 86%, acute respiratory viral infection – in 22% of women.

As a whole 27 mothers (77%) had spontaneous labor and 8 women (23%) had operative delivery. Accelerated labor was met in 3 women (9%), metrypercinesia was in 5 women (14%), and poor uterine contraction strength was in 3 women (9%). Foot or pelvic presentation were met in 7 cases (20%). Birth complications in the form of xerotocia were in 7 patients (20%), placental abruption and bleeding during childbirth was in 3 women (9%). Chorioamnionitis was diagnosed in 3 women (9%), pathology of the placenta and the umbilical cord was observed in 5 women (14%). Meconium in the water was in 7 women (20%).

The clinical course of VAP: The condition of all infants at birth was critical. All the children were born in asphyxia, including severe condition in 18 newborns. Severity of the condition at birth caused by the presence of respiratory, cardiovascular inefficiency and changes in the central nervous system (CNS). Indications for artificial pulmonary ventilation were respiratory disorders. They were caused by respiratory distress - syndrome (RDS) in 23 patients (66%), aspiration syndrome amniotic fluid in 9 patients (26%) and perinatal affection CNS in 3 women (9%). Artificial lung ventilation (ALV) was notices in 27 infants at birth (77%), ALV with 1.5 till 14 hours frequency was in 8 infants (23%). Duration of AVL was 16.9 ± 3.85 days in average.

Pneumonia was diagnosed on the 4 - 12 days of life (on the 4 - 12 days from the beginning of ALV). A severe form of pneumonia was in 29 infants (83%), medium clinical course was in 6 patients (17%), acute course was observed in 26 patients (74%), prolonged duration, i.e, disease duration for more than 50 days was in 9 patients (26%).

The beginning of pneumonia among the majority of infants refers to the first week of life (in 29 infants). The clinical picture was shown by onset of initial symptoms of infectious toxicosis by increasing respiratory failure, the growth and change in the nature of physical changes in the lungs. IVL parameters become insufficient for adequate ventilation. Ordinary, especially in infants with weight ≤ 1500g, during this period it was hearing a systolic heart murmur, there was a pulsation of the arteries, a tendency to tachycardia, indicating that the shunting of blood through the patent ductus arteriosus.

The majority of infants have a severe form of the disease which was developed florid symptoms of infectious toxicosis, respiratory failure and physical changes of the lung. Also the great initial weight loss pointed out on the severity of the condition and an unfavorable course of adaptation processes (more than 10% - 15% in 17 infants).

The changes in the lungs have always been two-sided and characterized by the presence of shortcut resonance, large, wet widespread and crepitant rattling in the lungs. Symptoms of tracheobronchitis with duration from 16 till 36 days were observed in most of infants.

The X-ray determined a double pulmonary involvement in most of cases (in 26 infants) in the form of macrofocal areas of pneumatization reduction, strengthen broncho-vascular drawing. Hydropic changes (segmental or general) along with focal shadows was determined in 20 infants. It was determined lobar atelectasis in 15 infants, atelectasis, pneumothorax in progression in 8 infants, appearance of interstitial air in 2 infants.

The changes in CNS stood out in the form of significant suppression of physiological reflexes, including those stable as sucking and nasopalpebral, motion activity and muscle tonus. Convulsive readiness and convulsion was observed in 5 infants.

Clinical manifestation of infection in acute toxicosis VAP characterized as hypothermia – in 7 infants (20%), single or short-term increase tº of body within 37.5 – 38.0˚C in 28 infants (80%). Grey color of the skin cover was in 24 infants (69%), «marbling» - in 22 (63%), hepatomegaly in 19 (54%), splenomegaly 9 (26%).

Changes in the respiratory system in the acute period VAP characterized by the presence of localized cyanosis in 34 infants (97%). Diffuse cyanosis was only in 1 infant (3%). Rapid breathing up to 51 - 80 per minute was in all infants. Increased rapidity of the chest

was observed in 21 infants (60%), shortening of percussion sound was in 34 (97%). Permanent heavy crackles in the lungs listened in 33 infants (94%), and dry rale only in 2 (6%). Tracheobronchitis occurred in 28 infants (80%). Pulmonary hemorrhage was diagnosed in 10 infants (29%).

The index of acid-base balance of blood indicated to the presence of metabolic acidosis in 11 infants (31%), respiratory acidosis in 14 (40%), mixed acidosis in 9 (26%) infants. The average pCO_2 in the blood was 69.3 ± 5.10 mm Hg and pO2 in blood 42.1 ± 1.6 (mm Hg). The oxygen-function blood was distressed. The level of Hb was lowered in 23 infants in average up to 103.2 ± 2.63 g/l and SaO_2 – 85 ± 2.2 % that fact was required strengthening artificial pulmonary ventilation (ALV).

Hemorrhagic syndrome was observed in 10 infants. It was shown in the form of pulmonary hemorrhage and driven by the presence of vasculitis purulonecrotic character. This information was obtained during pathomorphological research of dead infants.

There were changes in the cardiovascular system (CVS) in the form of weakening heart tones in 20 infants, an increase of its boundaries, the development of edema, tachycardia in 16 infants and bradycardia 4 infants.

The inflammatory process in the lung was combined with pathological symptoms from other organs and changes of laboratory parameters. In the peripheral blood during the acute period VAP was observed leukocytosis in 27 infants (77%) with increase of the number of white blood cells to 25.2 x 109 ± 2.01 / l. Neutrocytosis was in 28 infants (80%). Toxic granularity of neutrophils was observed in 7 infants (20%). Thrombocytopenia was occurred in 6 infants (17%).

Changes in blood biochemical values were as follows: Hypoproteinemia (protein content of < 55 g / l) was in 11 infants (31%), direct bilirubin> 10-15% of the total bilirubin was in 5 infants (14%). Twelve infants had an increased content of transaminases, including AST ≥ 50 mkmol / ml was in 12 infants (34%), ALT was ≥ 50 mkmol / ml in 5 infants (14%) increase or decrease of Na was in 4 (11%) increase or decrease of Ca were 2 patients (6%) infants. The level of C-reactive protein was raised in 3 infants and was 21.2 ± 3.8 mg / liter at the rate up to 6 mg / l.

It was revealed that 10 infants had renal dysfunction in the form of reduced diuresis in the first days of illness (< 2.1 ml / kg / hour), increasing of urea level ≥ 7mmol / l in 8 infants and creatinine ≥ 90 mmol / l - in 5 infants in the serum blood. Edematous syndrome was observed in 20 newborns. Pathological changes were observed in the general analysis of urine, in the form of proteinuria - in 14 infants, leukocyturia - in 11, microhematuria - in 8, bacteriuria - in 5, Candida-uria - in 1 infant.

The duration of pneumonia survivors was from 30 to 65 days and compound in average (M ± m) 39.2 ± 2.4 days. Severe condition lasted about 32.7 ± 376 days, including (phenomenon symptoms) the phenomena of infectious toxicosis – 26.5 ± 0.91 days. Respiratory failure was observed from 25 to 40 days (35.1 ± 2.81 days), rattling in the lungs was heard 29.5 ± 1.91 days. Staying in an oxygen hood was 22 ± 1.42 days. The necessity of infants staying in an incubator was 32.5 ± 3.92 days and the necessity of tube feeding was 28.08 ± 4.01 days. The positive dynamics of body weight was noted in average with 20.8 ± 2.0 days of life. Stationary admission time of infants in a hospital was 42.7 ± 2.82 bed days. As VAP complications were diagnosed bronchopulmonary dysplasia in 3 infants (9%) and sepsis - in 2 (6%) infants. There was a high mortality rate: 10 infants were died.

Microbiological studies: There were identified strains of *P. aeruginosa* among all the surveyed infants. It should be noted that *P. aeruginosa* as a pathogen of a primary infection was in 29 infants including as monoinfection in 23 infants and in association with other opportunistic pathogen in 6 infants. In conjunction with *P. aeruginosa* there were found *E. coli* in one infant, *Klebsiella pneumonia (K. pneumonia)* in 3 infants, *Staphylococcus aureus (S. aureus)* - in 2 infants. All these microorganisms were present in TBA in etiologically significant quantities.

P. aeruginosa as the germ of secondary infection was detected in six infants. There was a single change of the pathogen in 5 newborns (14%) among them. In the primary isolation there were marked *K. pneumonia* and *Enterobacter cloacae (E. Cloacae)* by 2 infants and *E. coli* in 1 infant. After 10 ± 0.81 days (with surge from 5 to 14 days) after the first survey was released in the etiologically significant amount of *P. aeruginosa*. It was accompanied by a deterioration of the clinical course of the disease. The change of pathogen was occurred twice in 1 infant. *K. pneumonia* was allocated in the primary isolation, *P. aeruginosa* was allocated after 7 days and *E.cloacae* was allocated after next 10 days.

All selected strains of *P. aeruginos*a with hemolytic properties, elastase and phospholipase activities.

There were identified hospital strains of *P. aeruginosa* in 10 premature infants. Based on the study of the genotype of these strains in PCR reaction with derivative of the primer short1 AATCGGGCTG it was revealed the identity profiles of amplicons with the size of more than 1000 bp - 200 bp DNA in the Group I of stains (4 strains). The other profiles of amplicon with the size 1000 bp - 300 bp were in the Group II of strains (two strains). There were revealed the identity of four strains for genotyping *P. aeruginosa* in another series of experiments, which were assigned to Group III. Thus, genotyping pointed to the presence of three different groups of strains of *P. aeruginosa* genotype. These three genetic groups of strains were different by antibiogram. Table 1 shows three phenotype *P. aeruginosa* strains in their sensitivity to antibiotics.

No	Antibiotics	I phenotype (n = 4)	II phenotype (n = 2)	III phenotype (n = 4)
1	Azlocillin	R	R	S
2	Imipenem/Cilastatin	R	SR	R
3	Meropenem	R	SR	R
4	Piperacillin	S	S	S
5	Piperacillin + Tasobactam	S	S	S
6	Carbenicillin	R	R	SR
7	Cefotaxime	R	R	SR
8	Ceftazidime	R	R	S
9	Ceftriaxone	R	R	S
10	Cefoperazone	R	R	S
11	Cefaclor	R	R	R
12	Doxycycline	R	R	R
13	Amicacin	S	S	S
14	Gentamicin	S	S	R
15	Kanamycin	R	R	R
16	Ofloxacin	S	SR	S
17	Ciprofloxacin	S	S	S
18	Chloramphenicol	R	R	R
19	Rifampicin	R	R	R
20	Tobramicin	R	R	S
21	Nitroxoline	R	R	S
22	Colistin	S	S	S
S – sensitive strain, R – resistant strain, SR – strain with intermediate sensitivity				

Table 1: *Antibiograms of three phenotypes hospital strains P. aeruginosa.*

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. aeruginos*a strain of type III - to 7 antibiotics.

The study of sensitivity to antibiotics of other 25 clinical strains of *P. aeruginosa* are shown in Table 2.

	Antibiotics	**S**	**R**	**SR**
1	Azlocillin	12	13	0
2	Meropenem	5	16	4
3	Imipenem/cilastatin	4	18	3
4	Piperacillin	25	0	0
5	Piperacillin + Tasobactam	25	0	0
6	Carbenicillin	0	25	0
7	Cefotaxime	5	18	2
8	Ceftazidime	13	11	1
9	Ceftriaxone	8	17	0
10	Cefoperazone	9	16	0
11	Cefaclor	0	25	0
12	Doxycycline	0	25	0
13	Amicacin	8	14	3
14	Gentamicin	2	21	2
15	Kanamycin	1	23	1
16	Ofloxacin	15	2	8
17	Ciprofloxacin	8	15	2
18	Chloramphenicol	0	25	0
19	Rifampicin	0	23	2
20	Tobramicin	10	23	2
21	Nitroxoline	4	26	5
22	Colistin	25	0	0
S – sensitive strain, R – resistant strain, SR – strain with intermediate sensitivity				

Table 2: *The sensitivity of clinical strains to antibiotics P. aeruginosa (n = 25).*

All 25 clinical *P. aeruginosa* strains had individual antibiogram and saved sensitivity to piperatsilinu, piperacillin / tazobactam and colistin. The other 13 strains were sensitive to azlocillin and ceftazidime. Thus all strains were multiresistant to a different spectrum of antibiotics.

K. pneumoniae strains showed resistance to most antibiotics and saved 100% sensitivity to imipenem / cilastatin, meropenem and amikacin, 4 strains were sensitive to ciprofloxacin, colistin, cefaclor. 2 – to rifampicin and 1 – to kanamycin. *E. coli* strain was sensitive to amikacin and β - lactam antibiotics pluripotent. Three strains of *E. cloacae* were retained sensitivity to cephalosporins of generation III, carbapenems, gentamicin, rifampin, 1 strain was sensitive to ciprofloxacin and rifampicin. Both *S. aureus* strains were sensitive to vanco-

mycin, linezolid, ciprofloxacin, cephalosporins of generation I-II, chloramphenicol and fusidin. One strain of *S. aureus* remained sensitive to lincomycin and fusidin and the second strain - to amikacin and gentamicin.

VAP treatment: Etiological treatment was conducted with sensitivity pathogens to antibiotics. Infants prescribed aminoglycosides alone or in combination with cephalosporins III generation, piperacillin, carbapenems. If there gram positive coccus administered vancomycin. Antibacterial therapy was combined with the appointment of immunoglobulin preparations for intravenous administration (Octagam, Pentaglobin or Intraglobin) during 3 - 5 days. All the infants also received pathogenetic therapy. Treatment of respiratory failure was carried out by the usage of mechanical artificial ventilation, then through a nasal catheter, mask and tent. The duration of ventilator apparatus was from 4 to 16 days.

Newborns also received infusion therapy. According to the testimony for the treatment of cardio - vascular insufficiency digoxin maintenance dose every 12 hours and Korglikon were ordered to infants. In case of hypovolemia and hypotension were used colloids and / or vasoactive drugs (dopamine and dobutamine).

In case of significant toxicosis in the acute phase of the disease and at high risk of developing bronchopulmonary dysplasia Dexamethasone was ordered at an initial dose of 0.5 mg / kg body weight per day followed by reduction.

In the treatment of necrotizing pneumonia with the high risk of developing DIC - syndrome, heparin was ordered at an initial dose of 100-150 IU / kg in three steps.

Infants received probiotics for the prevention and treatment of dysbiotic disorders in the gut.

Discussion

VAP development is closely related to the unfavorable conditions of intrauterine development, anamnesis burdened mothers, and complications in pregnancy and childbirth are:

Emergence of VAP in infants preceded syndrome of early postnatal adaptation of breath, cardiovascular and CNS.

The development of the disease was occurred in AVL conditions with high oxygen concentration and inspiratory pressure. The role of the ventilator is determined by the fact that when it is used actively as possible the penetration of micro-organisms in the lower respiratory tract from the external environment and from the upper respiratory tract of a newborn. The presence of an endotracheal tube into the airway off a significant portion of the local protection of the mucous membrane [1,4]. Furthermore, it is known that inhaled oxygen at high concentration may adversely affect the cells of the respiratory epithelium, making them more vulnerable to infection [7].

Strains of *P. aeruginosa* have pronounced toxic properties against the infant's body. Thus, phospholipase C, bacterial elastase, protease IV, alkaline protease, protease staphylolytic have destructive effects on the respiratory tract tissues and other organs. They contribute to the invasion of the pathogen in the blood and the spread of infection. Moreover, heat-stable and heat-labile hemolysins cause the destruction of red blood cells and disrupt their transport functions [4,9,14].

Pathogen features have their impact on the clinical manifestations of the disease. It is proceed with a serious infectious toxicity, long-term respiratory and cardio - vascular insufficiency, physical changes in the lungs, severe depression of the central nervous system, peripheral circulatory disorders, complications and high mortality.

Finding of one stationary 3 hospital strains *P. aeruginosa* among patients may point to their possible formation in the health facility or their getting from other clinics while transferring the newborn [9].

Conclusion

Thus, the violation of an early adaptation to the development of the infectious process causes the formation of severe VAP in the majority of preterm infants. The disease is preceded with severe forms of toxicity, trophic disorders, severe respiratory failure, bilateral, more common lesions of the lungs and bronchi, the frequent occurrence of atelectasis, pneumothorax, bronchopulmonary dysplasia and high mortality.

As a result of the conducted genotyping *P. aeruginosa* strains and antibiotic sensitivity studies was shown the presence of three different groups of strains that differed in antibiogram and genotype, indicating that they belong to the hospital strains.

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