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ORIGINAL ARTICLE

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The Results of Bacteriological Examination in Premature Infants with Neonatal Morbidity and Mortality

Agamurad A. Orazmuradov, PhD, ScD[1]; Igor N. Kostin, PhD, ScD[1]; Anastasiya N. Akhmatova, PhD[1]; Kemer F. Damirov[1]; Irina V. Savenkova[1]; Gayane A. Arakelyan[1]; Khalid Haddad[1]; Alexander M. Lopatin[1]; Sergey I. Kyrtikov[1]; Aleksey A. Lukaev, PhD[2*]

[1]Peoples' Friendship University of Russia (RUDN University), Moscow Russia
[2]Mytishchi City Clinical Hospital Mytishchi, Moscow Region, Russia

Abstract

The purpose of this study was to assess the results of bacteriological studies in children born prematurely and compare the received data with the detected neonatal morbidity.

Methods and Results: Our study included 227 pregnant women at gestational age of 28-36 weeks 6 days, and their newborns. Depending on the gestational age, they were divided into 3 groups. Group 1 included 73 women at gestational age of 28-30 weeks 6 days; Group 2 included 81 women at gestational age of 31-33 weeks 6 days, Group 3 included 73 women at gestational age of 34-36 weeks 6 days. All women underwent an assessment of vaginal microcenosis and the quantitative and qualitative composition of the biotope of the cervical discharge; the newborns underwent bacteriological examination of the auricle, pharynx and anus. Analysis of the results of bacteriological studies shows a significant growth of microorganisms in newborns from mothers of Group 1. The analysis of morbidity among premature infants showed that in each group there were 2 or 3 diseases, mainly of an infectious nature. The main proportion of morbidity (congenital pneumonia and infections of the perinatal period, diseases of the urinary system, neonatal jaundice of premature infants and cerebral ischemia) among newborns was found in Group 1, compared with Groups 2 and 3.

The analysis of the results obtained showed that the low birth weight in preterm labor correlated with the growth of *Staphylococcus epidermidis* in the throat of newborns. Neonatal jaundice of premature newborns was characterized by 100% detection of *Staphylococcus epidermidis* and *Serratia odorifera* in the anus swabs, and *Staphylococcus epidermidis* in swabs from the pharynx and ear. Congenital pneumonia positively correlated with the growth of *Staphylococcus epidermidis*, *E. coli*, *Candida spp*, *Enterococcus faecalis* in the throat swab. The deceased children had a co-infection.

***Conclusion*:** Our study identified the main microorganisms affecting both perinatal morbidity and neonatal mortality: *Staphylococcus epidermidis*, *Enterococcus faecalis*, *E. coli, Candida spp*. It is necessary to note the frequent identification of E. coli strains resistant to the main antibacterial drugs. (**International Journal of Biomedicine. 2020;10(4):357-361.**)

Key Words: premature infants • neonatal morbidity • microbiota • congenital pneumonia

Introduction

The high contamination of the genital tract of pregnant women with conditionally and absolutely pathogenic bacterial and viral microbiota is a factor of high risk for premature birth, and the shorter the gestation period, the higher the frequency of vaginal biotope disorders. The risk of early preterm birth correlates with the presence of infection in the mother. It is the data of the first bacteriological culture that seem important, since after a course of antibacterial therapy, the subsequent results of bacteriological cultures are not very informative for an adequate assessment of the vaginal microbiota.[1]

According to the American Association of Obstetricians and Gynecologists, the incidence of membranes rupturing before 37 weeks' gestation ranges from 5% to 35% and complicates 2%-4% of singleton pregnancies.[2,3] S. Mamedova, studing the nature and degree of contamination

*****Corresponding author:** Aleksey A. Lukaev, PhD. Mytishchi municipal clinical hospital, Mytishchi, Moscow Region, Russia E-mail: aleksei_lukaev@mail.ru*

of amniotic fluid, has found premature rupture of membranes due to monoinfection, dual infection, and triple infection in 24.4%, 63.3%, and 12.2% women, respectively.[4]

The purpose of this study was to assess the results of bacteriological studies in children born prematurely and compare the received data with the detected neonatal morbidity.

Materials and Methods

This research was carried out in 2017–2018. Our study included 227 pregnant women at gestational age of 28-36 weeks 6 days, and their newborns. Depending on the gestational age, they were divided into 3 groups. Group 1 included 73 women at gestational age of 28-30 weeks 6 days; Group 2 included 81 women at gestational age of 31-33 weeks 6 days, Group 3 included 73 women at gestational age of 34-36 weeks 6 days.

All women underwent an assessment of vaginal microcenosis and the quantitative and qualitative composition of the biotope of the cervical discharge; the newborns underwent bacteriological examination of the ear, pharynx, and anus.

To analyze the nature of the quantitative and qualitative composition of the biotope of the cervical discharge, a tampon-probe and test tubes with a transport medium ("Medical Wire & Equipment," England) were used. Seeding was performed on a series of nutrient media to isolate and cultivate various groups of microorganisms: 5% blood agar based on Brucella agar with the addition of vitamin growth factors to isolate and cultivate anaerobes, mannitol salt agar to isolate and cultivate gram-negative bacteria, and Saburo medium to isolate and cultivate fungi. Blood agar media were cultivated in a thermostat with a high content of carbon dioxide (5-10%). To cultivate anaerobes we used anaerostats (Becton Dickinson, USA). The isolated microorganisms were identified and their sensitivity to antibacterial drugs was determined using the Witek bacteriological analyzer. The results obtained were recorded in accordance with the NCCLS standards (1999–2000). The number of isolated microorganisms was determined by the density of their growth on the sectors of the agar plate.

Bacteriological study of auricular secret and pharynx

The material obtained and transported to the laboratory was seeded on Petri dishes with 5% blood agar, chocolate agar, and yolk-salt agar, on Endo medium, Saburo medium, and in a tube with glucose broth. Seeding on dense nutrient media was carried out metered (according to Gould), which made it possible to quantify the number of grown colonies. Seedings were incubated at 37°C for 24–48 hours, and were examined daily. Plates with 5% blood agar were incubated under conditions with a high CO_2 content. With the appearance of growth on nutrient media, we counted colonies of various morphologies, taking into account their ratio and species identification of microorganisms, as well as determining their sensitivity to antibacterial drugs. A negative result of the study was issued in the absence of growth on all nutrient media for 72–96 hours.

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the RUDN University Ethics Committee. Written informed consent was obtained from all participants.

Statistical analysis was performed using the Statistica 8.0 software package (StatSoft Inc, USA). Baseline characteristics were summarized as frequencies and percentages. Group comparisons with respect to categorical variables are performed using chi-square test. The Spearman's rank correlation coefficient (r_s) was used to determine the strength and direction of the relationship between two variables. The odds ratio (OR), its standard error and 95% confidence interval (CI) were calculated. Logistic regression was used to model dichotomous outcome variables. A value of $P<0.05$ was considered statistically significant.

Results

Analysis of the results of bacteriological studies shows a significant growth of microorganisms in newborns from mothers of Group 1 (Table 1). In general, there were statistically significant differences in the detection of microbial growth between groups, with statistically significant differences between Groups 1 and 2 (P=0.0068), Groups 1 and 3 (P<0.001), and Groups 2 and 3 (P=0.0257).

Table 1.
The results of bacteriological studies in newborns

Detection of microbial growth	Group 1 (n=73)	Group 2 (n=81)	Group 3 (n=73)
Throat swab	48 (65.8%)	41 (50.6%) P_{1-2}=0.0576	33 (45.2%) P_{1-3}=0.0125
Anus swab	45 (61.6%)	46 (56.8%)	25 (34.3%) P_{1-3}=0.0009 P_{2-3}=0.0051
Ear swab	42 (57.5%)	40 (49.4%)	30 (41.1%) P_{1-3}=0.0470
Growth of different microbial strains in low titers (throat, anus, ear)	63 (86.3%)	58 (71.6%) P_{1-2}=0.0264	43 (58.9%) P_{1-3}=0.0002 P_{2-3}=0.0976

The analysis of morbidity among premature infants showed that in each group there were 2 or 3 diseases, mainly of an infectious nature: congenital pneumonia and infections of the perinatal period, diseases of the urinary system, neonatal jaundice of premature infants and cerebral ischemia. The main proportion of morbidity among newborns was found in Group 1, compared with Groups 2 and 3 (Table 2).

According to the results of bacteriological examination in women, mainly mono- and dual microorganisms were found, less often a combination of 3 and even less often 4 microorganisms.

The analysis of the results obtained showed that the low birth weight in preterm labor correlated with the growth of *Staphylococcus epidermidis* in the throat of newborns (r_s=0.411, P<0.001). Neonatal jaundice of premature newborns was characterized by 100% detection of *Staphylococcus epidermidis* and *Serratia odorifera* in the anus swabs, and *Staphylococcus epidermidis* in swabs from the pharynx and ear (Table 3).

Congenital pneumonia positively correlated with the growth of *Staphylococcus epidermidis* (r_s=0.722, P<0.001), *E. coli*(r_s=0.416, P<0.001), *Candida spp.* (r_s=0.334, P<0.001), *Enterococcus faecalis* in the throat swab. A direct correlation was found between neonatal morbidity and the growth of *E. coli* in the throat of newborns (r_s=0.703, P<0.001) and *Serratia odorifera* and *Candida spp.* (r_s=0.314, P<0.05) in the anus swabs.

The deceased children had a co-infection (Table 4, 5). For example, in a postnatally dead child from Group 1, we found a moderate growth of *Staphylococcus aureus* in the throat swab, an abundant growth of *Staphylococcus haemolyticus* in the anus swab and an abundant growth of *Staphylococcus epidermidis* in the ear swab. In Group 2, in 2 dead children, the growth of *E. coli*, resistant to antibiotic therapy, was determined in the throat swab.

Using binary logistic regression analysis, a reliable model (P<0.001) was obtained, and based on this the most significant bacteria were identified (Table 6), the detection of which allows predicting an unfavorable pregnancy outcome and the presence of neonatal morbidity.

Table 2.

The main diseases and congenital malformations of children identified in the maternity hospital

Neonatal morbidity	Group 1 (n=73)	Group 2 (n=81)	Group 3 (n=73)
Congenital pneumonia	35 (47.9%)	17 (21.0%) P_{1-2}=0.0004	9 (12.3%) P_{1-3}=0.0000
Other infections of the perinatal period	21 (28.8%)	11 (13.6%) P_{1-2}=0.0204	6 (8.2%) P_{1-3}=0.0014
Low birth weight	9 (12.3%)	22 (27.2%) P_{1-2}=0.0219	34(46.6%)* P_{1-3}=0.0000 P_{2-3}=0.0124
Intraventricular hemorrhage	14 (19.2%)	9 (11.1%)	1 (1.4%) P_{1-3}=0.0004 P_{2-3}=0.0143
Cerebral ischemia	63 (86.3%)	43 (53.1%) P_{1-2}=0.0000	31 (42.5%) P_{1-3}=0.0000
Diseases of the urinary system	17 (23.3%)	6 (7.4%) P_{1-2}=0.0058	3 (4.1%) P_{1-3}=0.0008
Neonatal jaundice of premature infants	26 (35.6%)	42 (66.7%) P_{1-2}=0.0428	32 (43.8%) P_{2-3}=0.3202
Congenital atresia of the esophagus	4 (5.5%)	1 (1.2%)	1 (1.4%)
Hemorrhagic disease	3 (4.1%)	1 (1.2%)	-
Total	73 (100.0%)	54 (66.7%) P_{1-2}=0.0000	37 (50.7%) P_{1-3}=0.0000 P_{2-3}=0.0440

Table 3.

Neonatal morbidity and identified microorganisms in premature newborns

Neonatal morbidity	Throat swab	Anus swab	Ear swab
Congenital pneumonia (n=52)	*Staph. epidermidis* - 42.9%; *Proteus mirabilis* -17.3% *E. coli* - 32.7%; *Enterococcus faecalis* - 28.6% *Candida spp.*- 30.8%; *Staph. warneri* - 26.9%	*Staph. saprophyticus* - 25%	
Other infections of the perinatal period (n=38)	*Staph.epidermidis* -10.5% *Staph.saprophyticus* - 34.2% *Str.viridans* -10.5%	*Serratia odorifera* - 26.3% *Staph. epidermidis* - 31.6%	*Enterococcus faecalis* -13.2%
Low birth weight (n=65)	*Staph. epidermidis* -76.9% *Enterococcus faecalis* - 49.2%	*Staph. saprophyticus* - 26.2%	
Intraventricular hemorrhage (n=25)	*Staph. epidermidis* -24%; *Proteus mirabilis* - 24% *Enterococcus faecalis* - 25%; *Candida spp.*- 50% *Str.viridans* -20%		*Staph. epidermidis* -25%
Cerebral ischemia (n=137)	*Str.viridans* - 28.5%; *Staph. warneri* - 14.3% *Staph. aureus* - 8.8%; *Staph. epidermidis* - 27% *Proteus mirabilis* - 13.9%	*E. coli* - 25.5% *Staph. epidermidis* - 28.6% *Staph. haemolyticus* - 14.3% *Serratia odorifera* - 21.4% *Corynebacterium spp.* - 7.1%	*E. coli* -14.3% *Enterococcus faecalis* -21.4% *Staph. epidermidis* -42.9%
Diseases of the urinary system (n=26)	*Staph. epidermidis* -46.2%; *Staph. warneri* -34.5% *Enterococcus faecalis* -15.4%	*E.coli* - 23.1%; *Staph. epidermidis* -26.9% *Serratia odorifera* - 34.6%	*Enterococcus faecalis* -26.9% *Staph. epidermidis* - 23.1%
Congenital atresia of the esophagus (n=6)			*Serratia marcescens* -50%
Neonatal jaundice of premature infants (n=100)	*Staph. epidermidis* - 50%; *Staph. saprophyticus* -25% *Enterococcus faecalis* -20%	*Staphylococcus epidermidis* - 25% *Serratia odorifera* - 30%	*Staph. epidermidis* – 30%
Hemorrhagic disease (n=4)	*Serratia marcescens* -25%; *Proteus mirabilis* - 50%		

Table 4.
Frequency of bacterial growth and neonatal mortality

	Group1 (n=73)	Group 2 (n=81)	Group 3 (n=73)
Neonatal mortality	8 (11.0%)	6 (7.4%)	-
Throat swab	7 (87.5%)	4 (66.7%)	-
Anus swab	4 (50%)	2 (33.3%)	-
Ear swab	3 (37,5%)	1 (16.7%)	-
Detection of microbial growth	8 (100%)	5 (83.3%)	-

Using the binary logistic regression method, the probability of developing diseases in children was determined, based on the data, by the formula:

$$p = \frac{1}{1+e^{-z}}$$

where: *e* is the base of the natural logarithm, approximately equal to 2.718

$z = -7.172 + 0.314 \times X1$

In the previous studies,[1,5] we showed the main risk factors leading to premature birth: early sexual debut, inflammatory diseases of the urinary organs, infectious factors, including sexually transmitted infections, reproductive losses in history, anemia, etc. In the present study, the results obtained indicate a significant negative effect of opportunistic flora on perinatal outcomes. Our study identified the main microorganisms affecting both perinatal morbidity and neonatal mortality: *Staphylococcus epidermidis*, *Enterococcus faecalis*, *E. coli*, *Candida spp*.

Table 5.
The results of bacteriological examination in the deceased children

	Group 1 (n=8)	Group 2 (n=6)
Throat swab		
Staph. epidermidis	4 (50%)	2 (33.3%)
E.coli	3 (38%)	2 (33.3%)
Enterococcus faecalis	2 (25%)	1 (16.7%)
Candida spp.	2 (25%)	1 (16.7%)
St.saprophyticus	-	1 (16.7%)
Neisseria spp	-	1 (16.7%)
Staph..aureus	2 (25%)	1 (16.7%)
Proteus mirabilis	1 (13%)	-
Ear swab		
Staph. epidermidis	2 (25%)	2 (33.3%)
E.coli	1 (12.5%)	1 (16.7%)
Enterococcus faecalis	1 (38%)	1 (16.7%)
Staph..aureus	2 (25%)	1 (16.7%)
Proteus mirabilis	1 (12.5%)	-
Anus swab		
Staph. haemolyticus	1 (12.5%)	-
E.coli	1 (12.5%)	1 (16.7%)
Enteroccocus faecalis		1 (16.7%)
Serratia marcescens		1 (16.7%)
Proteus mirabilis	1 (12.5%)	-

Table 6.
Mathematical model for the main microorganisms affecting perinatal morbidity

Statistics	Const.B0	Staph. epirmidis	Enteroccocus faecalis	E.coli	Candida spp
Estimate	-2.507	0.412319	0.349	0.4228	0.034
Standard Error	1.5042	0.167	0.167	0.1367	0.012
t(129)	-0.00783	2.46511	1.995028	3.091231	2.86897
P-value	0.9938	0.0150	0.0496	0.0024	0.0048
-95%CL	-2.98795	0.081387	0.00251	0.152412	0.05832
+95%CL	2.964407	0.74325	0.701628	0.693305	0.21071
Wald Chi-square statistics	6.12E-05	6.076768	3.861334	9.555711	8.230992
P-value	0.993756	0.013702	0.04942	0.001995	0.004121
Odds ratio (unit ch)	0.988297	1.510316	1.418444	1.526318	1.366072
-95%CL	0.050391	1.084791	1.07496	1.164639	1.043348
+95%CL	19.38321	2.102759	2.017034	2.000315	1.989344

It is necessary to note the frequent identification of *E. coli* strains resistant to the main antibacterial drugs. These results will help to predict the risk of premature birth in a timely manner, and to carry out adequate antibiotic therapy, thus increasing the possibility of a favorable outcome of premature birth and prolongation of pregnancy with timely successful treatment.

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Competing Interests

The authors declare that they have no competing interests.

References

1. Orazmuradov AA, Kostin IN, Apresyan SV, Parygina AN, Gavrilova AA, Arakelyan GA, Savenkova IV, Lukaev AA. The Results of Microbiological Investigations into Preterm Labor.International Journal of Biomedicine. 2019;9(2):144-149. doi: 10.21103/Article9(2)_OA13

2. Egorova AT, Ruppel NI, Moiseenko DA. [The course of pregnancy and childbirth in spontaneous multiple and singleton pregnancy]. Scientific Bulletin of Belgorod State University. Series: Medicine. Pharmacy. 2015;30(10):75-80. [Article in Russian].

3. ACOG Committee on Practice Bulletins-Obstetrics. ACOG Practice Bulletin No. 80: premature rupture of membranes. Clinical management guidelines for obstetrician-gynecologists. Obstet Gynecol. 2007 Apr;109(4):1007-19. doi: 10.1097/01.AOG.0000263888.69178.1f.

4. Mamedova SN. [Characteristics of the degree of contamination of amniotic fluid in case of premature rupture of the amniotic membranes, as well as mucus of the upper respiratory tract in newborns with RDS, born to mothers with premature rupture of the membranes in the conditions of the ROC in Baku]. In the collection: Global science. Development and novelty. Collection of scientific papers on materials X International Scientific Conference. L-Zhurnal. 2019:50-54. [Article in Russian].

5. Orazmuradov AA, Soyunov MA, Efendieva ZM, Lukaev AA, Gradova IE, Mukhtarova AV, Kaplina IN. Influence of Perinatal Risk Factors on Premature Labor Outcome. International Journal of Biomedicine. 2016;6(3):199-201. Doi: 10.21103/Article6(3)_OA8