

Analysis of microorganisms isolated from cervical canal of pregnant women giving birth prematurely in Poland

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ABSTRACT

Purpose: Evaluation the rate of bacterial colonization of the genital tract among a large group of Polish women and the relationship between the types of bacteria from the cervical canal of pregnant women and preterm delivery.

Materials and methods: 1084 pregnant women were taken swab from the cervical canal, 127 born prematurely. Bacteriological studies were performed to identify aerobic and anaerobic bacteria, and fungi.

Results: Colonization of birth canal with *Enterobacteriaceae* was not associated with preterm

delivery statistically. Among women giving birth prematurely high virulence bacteria were detected statistically often ($p = 0.047$). *Enterococcus faecalis* infections, which occurred significantly more frequently in pregnancies complicated preterm premature rupture of membranes (pPROM).

Conclusions: The bacteria of low virulence, physiologically colonize the gastrointestinal tract is not an etiological factor of preterm labor and pPROM.

Key words: bacteria, pregnant, women, preterm, delivery

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INTRODUCTION

Intrauterine infection is described in the literature worldwide. In most countries, it is a common cause of preterm birth and intrauterine fetal death. It is known that the frequency of infection depends on the gestational age. Birth before 28 week of gestation is strongly associated with infection of the amniotic fluid, while the subsequent premature births and births at the time seem to be less correlated with infection [1,2]. Therefore, it is vital to early diagnosis of infection, especially to take an appropriate decision as to the time of delivery or the use of antibiotics and corticosteroids.

Most microorganisms colonizes the cervical canal and uterine cavity during pregnancy ascending from the vagina. However, note that there are microorganisms that colonize the cavity of the uterus before pregnancy. Many of them infect the amniotic fluid by the intact membranes or immediately after the rupture, and then infect the fetus. A review of the literature suggests that bacterial vaginosis and other ascending bacterial infection, usually the rupture of membranes, cause infection of chorion, decidua, or amnion, and their inflammatory response [3,4,5,6]. In about half the cases, the infection is confined to the chorion, but in many other organisms cross the barrier of membranes and infect the amniotic fluid. Only in exceptional cases, these microorganisms cause infection of fetus. During pregnancy or birth mother may pass to the fetus many microorganisms that colonize the birth canal, mainly β -hemolytic streptococcus, the bacteria from the Enterobacteriaceae group such as *Escherichia coli* or *Enterococcus faecalis*, then *Staphylococcus aureus*, anaerobes, *Mycoplasma*, *Chlamydia* and fungi [2,6,7-10]. In most of microorganisms are characterized by a low virulence, and even during the birth, most pregnant women do not develop the classic symptoms of infection. Instead of signs of intrauterine infection may occur premature uterine contractions or rupture of membranes. Bacteria such as group B streptococci, *Escherichia coli*, *Klebsiella* and staphylococci may even pass through intact membranes and cause intrauterine infection [2,8]. But when an infection develops, anti-inflammatory response is faster, more abrupt and usually end up giving birth in a few hours or days.

So far there was no comprehensive research in Poland described the rate of bacterial colonization of the genital tract among a large group of Polish women and the relationship between the types of bacteria from the cervical canal of pregnant women and preterm delivery.

The aim of the study was to estimate the incidence rate of microorganisms, their identification and determination the effect of the

bacteria colonizing the cervix canal in women delivering prematurely and at time.

MATERIAL AND METHODS

Tests were carried out in the Public Hospital of Witold Orłowski in Warsaw in the years 2005 - 2007, after approval by the Bioethics Committee No 6/2006. Prospective analysis included 1054 pregnant women, hospitalized at the Department of Obstetrics and Gynecology. Nine women whose children died because of congenital abnormalities or extreme prematurity in the first three days of life were excluded. In addition, 21 pregnant women whose children were transported to specialize in pediatric centers due to congenital anomalies requiring urgent surgical treatment were excluded from the analysis.

After assumption of a sterile speculum, secretion from the external ostium of the cervix canal was collected in the transport medium or taken into account the results of microbiological samples up to 14 days before parturition. Bacteriological studies were performed to identify aerobic bacteria, anaerobic and fungi. Within 24 hours the samples were placed on appropriate media and incubated at 37°C for 24-48 hours. Then, the sensitivity to antibiotics of isolated pathological microorganisms was determined by diffusion method.

The results were analyzed statistically based on STATA 8.0. In the analysis of statistical differences was used test Person Chi2 and test based on an analysis of variance. The level of statistical significance was $p < 0.05$.

RESULTS

2472 women delivered in 2005 and first half of 2006 in the Department of Obstetrics and Gynecology. Among them, 1084 pregnant women were taken a swab from the cervical canal.

Age of women ranged from 15 to 51 years, average 29.12 (median 29, SD 4.562). The mean age of primipara was 27.71 (15 - 43 years), the mean of women delivering second time 38.48 (19 - 51 years) and the delivering third time 33.41 (23 - 44 years). The study population there were 59.3% of primipara, 38.81% of multipara and 1.89% of multiple multipara (>3 births), The average pregnancy rate was 1.727 (median 1, SD 0.942), mean parity 1.51 (median 1, SD 0.716). In the study group there were 1030 singleton pregnancies, 22 twin pregnancies and 2 triplets.

Gestational age ranged from 23 to 42 weeks of gestation, mean 38.542 (median 39, SD 2.584). There was 1.12% of pregnancies under 28-week gestation; 2.93% pregnancies from 28 to 33-week gestation; 7.98% pregnancies from 34 to 37

weeks gestation, and 87.95% of pregnancies more than 37 weeks of gestation. 60.06% of pregnant women took physiological delivery, in 38.99% of cases Caesarean section was performed, and 0.95% were used vacuum extractor.

In the study group 57.21% of pregnant women had normal microbial culture results from the external ostium of the cervix canal - only the natural flora strains: *Lactobacillus* spp., *Propionibacterium* spp, *Peptococcus* spp. Figure 1

shows the percentage distribution of microorganisms cultured from the cervix in the study population.

Among the pathogenic flora dominated: 15.46% *Enterococcus faecalis*, *Streptococcus agalactiae* 14.89% and *Candida* 13.28%. *Klebsiella* spp. - 2.058% dominated among the high virulence species. Most are grown together: *E. coli* and *E.faecalis*, *E.faecalis* and *S.agalactiae* and *E.faecalis* or *S.agalactiae* of *Candida* spp.

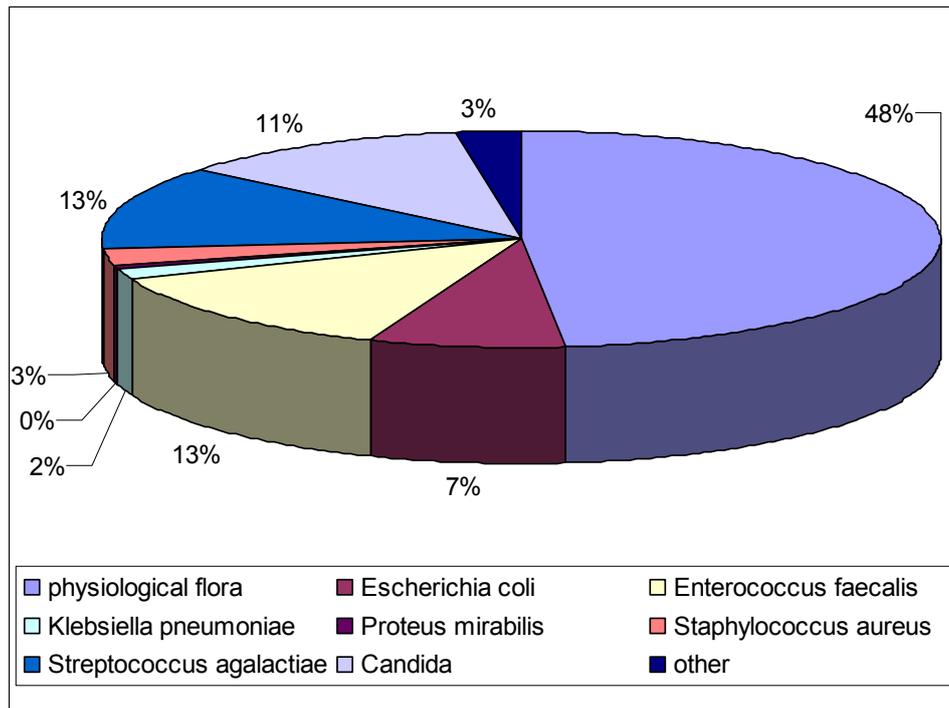


Figure 1. The distribution of microorganisms cultured from the cervical canal.

In the study group, 127 pregnant women (12.05%) children born prematurely. The average gestational age at preterm delivery was 33.13 weeks (median 35, range from 23 to 36 weeks of gestation). 50.39% women born physiological, and 49.61% underwent caesarean section, mainly because of multiple pregnancy, pathology of the placenta, pregnancy-induced hypertension and the state after cesarean section.

Figure 2 compares the presence of natural flora in the cervical canal in women giving birth pathological (46.5% vs 53.5%). An analogous, though the slightly higher proportion occurred among women delivering preterm, less than 37 weeks of gestation (42.5% vs 57.48%). No significant correlation between the week of delivery and the result of inoculation with cervical canal was found.

Figure 3 shows the relation between the cervical bacteria during pregnancy and preterm delivery. Among women giving birth prematurely 14.17% were infected with β -hemolytic

streptococci in the birth canal and 85.83% non-infected, by analogy, 16.29% and 83.71% among women in labor at the time. There was no significant variation rate of infection among pregnant women below and above 37 weeks of pregnancy ($p = 0.542$).

Analyzing *Enterococcus faecalis* colonization, the percentage of infected women delivering at time was 17.04% and 85.96% uninfected. Lower proportion was observed among women giving birth prematurely - by analogy, 11.02% vs 88.98%. There was no significant difference was the percentage of *Enterococcus faecalis* infections (Pearson chi2 test = 2.9649, $p = 0.085$). When considering the percentage of *Escherichia coli* infection proved a slight increase among women delivering below 37 weeks of pregnancy compared with women delivering at the time: 9.45% and 90.55% vs 8.95% and 91.05%. However, there was no significant variation rate of infection of *Escherichia coli* (Pearson Chi2 test = 0.0334, $p = 0.855$).

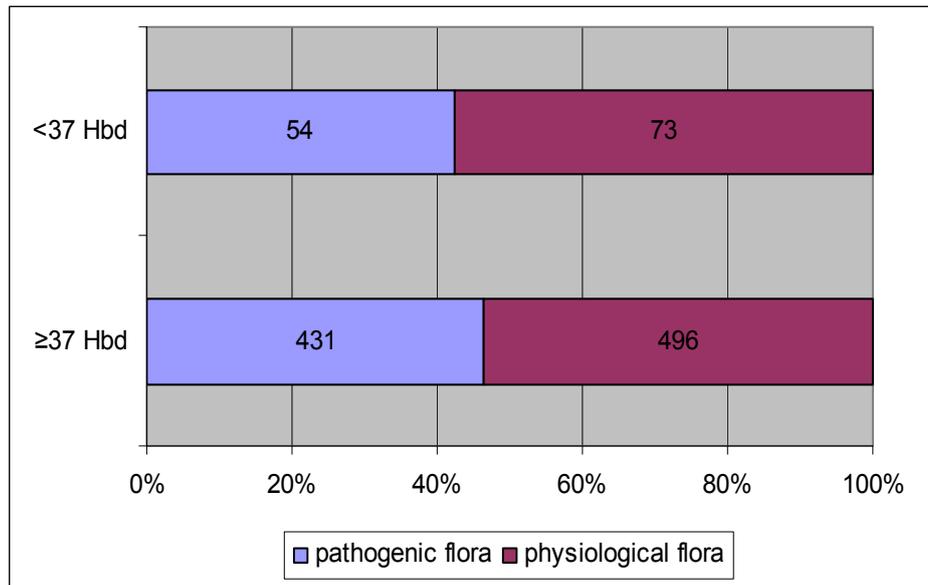


Figure 2. The presence of physiological flora in the cervical canal in women giving birth prematurely and the time.

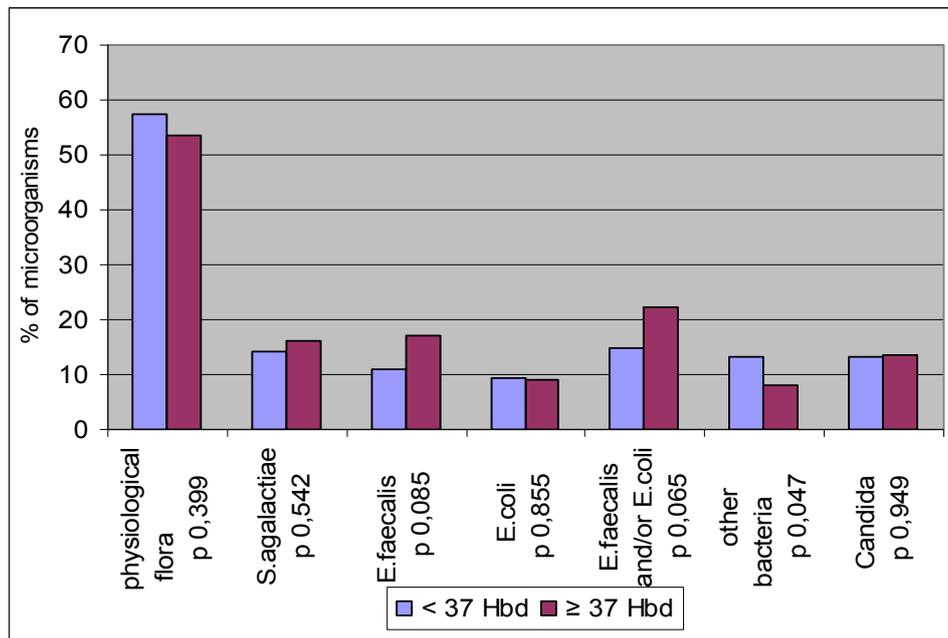


Figure 3. The relationship between the bacterial flora of the cervical canal and preterm delivery.

Taking into account both the bacteria belong to *Enterobacteriaceae* group, preterm delivery was associated with 14.96% of infection *E. coli* and / or *E. faecalis* comparing the birth at time with a greater percentage of 22.11% of infections.

There was no significant differences in statistics: Pearson Chi2 test = 3.4156, p = 0.065.

Fungal infection with *Candida* was diagnosed in 13.38% women in premature delivery and in 13.59% deliver at the time, while finding no

significant statistical differences (Pearson χ^2 test = 0.0041, $p = 0.949$).

Then the presence of other than the above-mentioned pathogenic bacteria in the cervical canal of pregnant women was analyzed. Among women giving birth prematurely was 13.38% infected and 86.61% uninfected, while a lower rate of infection in pregnancy more than 37 weeks was showing, respectively 8.09% and 91.9%. The significant differentiation rate of infection among women below and above 37 weeks gestation were found (Pearson χ^2 test = 3.9312, $p = 0.047$). Among women giving birth prematurely, *Klebsiella pneumoniae* was detected in 6 cases, in swabs taken from the cervical canal (35.29%), *Corynebacterium* infection has been detected in two cases and one case of: *Aerococcus viridans*, *Eubacterium*, *freundii* *Citrobacter*, *Citrobacter kosevi*, meticolin-sensitive *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus* and alpha-hemolic oralis *Staphylococcus*.

During the study population of women giving birth prematurely were observed statistically significant differences of the cervical canal flora depending on the duration of pregnancy. 56% of the physiological flora was diagnosed in the swab from the cervical canal of 25 pregnant women with early preterm delivery or until 30 weeks of pregnancy, and 44% of pathogenic microorganisms, mainly *Candida spp.*, *Klebsiella pneumoniae* and *Enterobacteriaceae*. In 101 women with late preterm birth after 30 weeks of pregnancy, the pathogenic flora in the cervical canal were detected in 36.63%: *Streptococcus agalactiae*, *Candida spp.*, and bacteria of the *Enterobacteriaceae* group. 63.37% of the results were correct.

Premature rupture of membranes in preterm pregnancy occurs in 2.9%- 3.5% of all pregnancies and is the most common symptom preceding preterm delivery. Chorioamnionitis caused by bacteria that cause the weakening of the membranes is considered as one of the reasons for this state. In the whole study group, 192 pregnancies (18.22%) were complicated by the premature rupture of membranes, of which 22.39% (43 cases) of pregnancies was less than 37 completed weeks of gestation.

The statistical occurrence of particular bacteria in pregnancy complicated by PROM 37Hbd above and below was compared in this part of the study. Among all pregnancies with preterm premature rupture of membranes, 22.39% were completed below 37 weeks of gestation. 62.79% of physiological flora isolated from the cervical canal was detected in the pregnancy associated with

pPROM. In pregnancies above 37-week gestation complicated by PROM rate amounted to 52.35%. At the significance level of 0.05, there was no significant difference between the percentage of cultures with physiological flora in pregnancies complicated with preterm rupture of membranes.

Figure 4 compares the flora of the cervical canal in pregnancy complicated with premature rupture of membranes above and below 37 weeks. Of all the preterm pregnancies complicated with premature rupture of membranes, *Streptococcus agalactiae* was detected in the cervical canal in 13.95%. This is a slightly lower percentage compared to pregnancies completed on time - 19.46%, however, there were no significant differences ($p=0.340$). *Enterococcus faecalis* infection was diagnosed at a much higher percentage - 18.79% in full-term pregnancies presenting with premature rupture of membranes in comparison with preterm pregnancies - 4.65%, while finding statistically significant differences ($p = 0.038$). Analyzing the percentage of infection with *Escherichia coli* similarly was demonstrated a slight increase in full-term pregnancies among 8.72% compared to 4.65% of premature pregnancies. However, there was no significant difference was the percentage of *Escherichia coli* infection ($p=0.411$). Considering the fungal infection, *Candida* infection rates were comparable in premature and completed on time pregnancies running with premature rupture of membranes - 6.98% and 8.72% ($p = 0.69$).

Then the presence of other than the above-mentioned pathogenic flora of the cervical canal in pregnancy complicated with premature rupture of membranes below and above 37 weeks of gestation was analyzed. 13.95% of infected women was among giving birth prematurely, and more than 37 weeks gestation 6.71% respectively. Significant differentiation rate of infection among women below and above 37 weeks gestation ($p = 0.05$) was found.

Premature rupture of membranes in preterm pregnancy was diagnosed in 43 (4.08%) pregnancies in this study. The presence of specific bacteria in the pregnancy complicated with premature rupture of membranes before the date of birth and normal pregnancy from 37 to 42 weeks of gestation was compared statistically. Figure 5 compares the microbial flora of the cervical canal in pregnancy complicated by preterm premature rupture of membranes with physiologic pregnancy from 37 to 42 weeks of gestation except in cases complicated by PROM (Fig. 5).

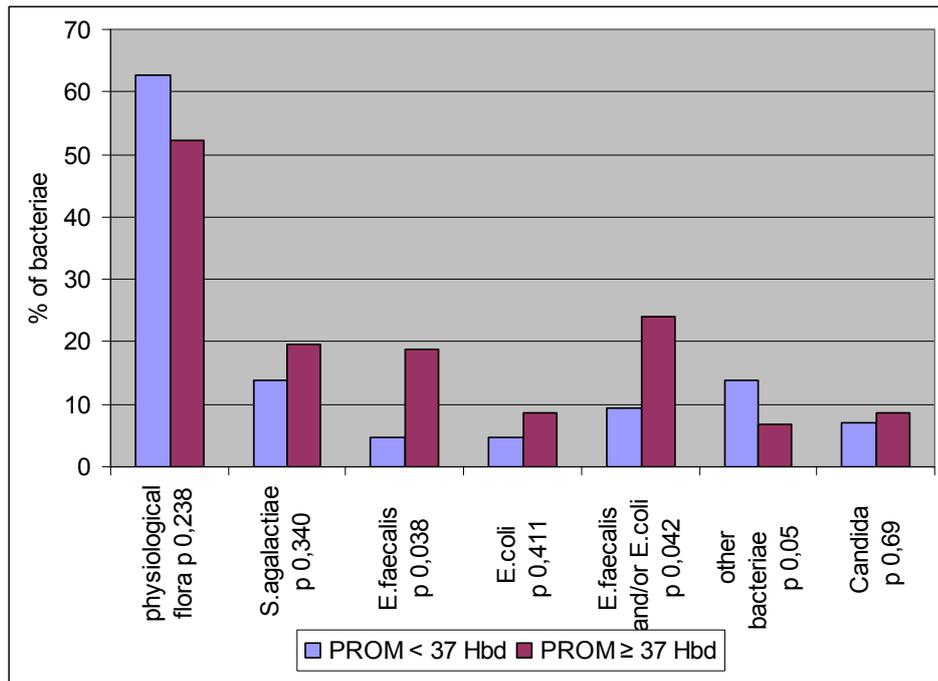


Figure 4. The differences between the flora of cervical canal in pregnancy complicated with premature rupture of membranes above and below 37 weeks of gestation.

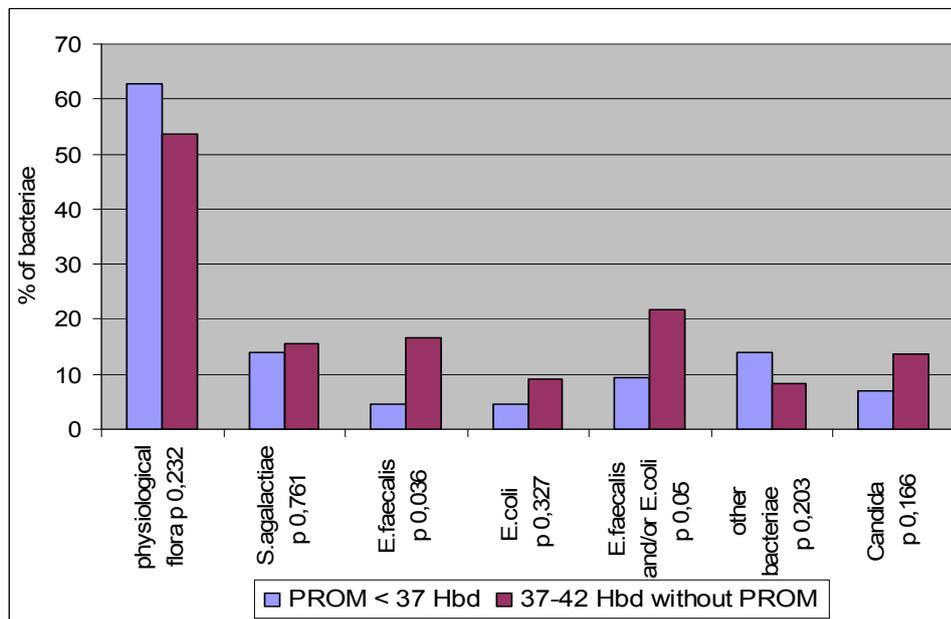


Figure 5. Microbial flora of cervical canal in pregnancy complicated by premature rupture of membranes below 37 weeks of gestation compared with physiologic pregnancy from 37 to 42 weeks.

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DISCUSSION

Pathogenic microorganisms more often found in the vagina of women delivering prematurely with or without rupture of membranes. Premature birth is one of the major problems of modern obstetrics, causing up to 70% mortality and more than half of neonatal neurological damage.

Despite many years of research the etiology of preterm delivery remains to be unraveled. Several studies confirm the complexity of reasons, both from the mother's side or the blastocyst and depending on the external environment [3,11]. Approximately 10% of all births are premature birth and extreme prematurity or birth before the age of 32 weeks of pregnancy concerns, depending on the country, from 0.9% to 1.9% of all births. Morbidity and mortality rates applicable from 1% to 2% of newborns who were born below 32 weeks of gestation, and whose mass is less than 1500g. About 20% of pregnancies are completed before by the decision of obstetricians due to maternal or fetal indications.

The rate of preterm birth in Western Europe and the U.S. has not changed in decades despite considerable progress in perinatal medicine, while the survival rate of preterm born infants has increased, so that 80% of premature infants weighing between 500g and 1000g is experiencing.

According to data from the National Health Fund in Poland rate of preterm birth decreases from year to year, but the distance that separates us from the most developed European countries is still significant. In our country, in 2000, the birth rate of newborns with low birth weight below 2500g was 6.1%, while extreme prematurity affected approximately 1.5%. Nearly 80% of perinatal mortality relates to preterm infants. Limiting the number of preterm deliveries and prolonged duration of pregnancy has a significant effect on improving infant mortality rates, further important benefit is to reduce the risk of neurological complications and abnormal psychomotor development in the future.

Intrauterine infection is an important cause of preterm delivery quoted in the literature [1,5,12-14]. The relationship between maternal birth canal infection and preterm delivery is not continuous and constant during pregnancy. Infection is a rare

Analyzing the various species of bacteria, no significant statistical differences in the test of significance of the difference fraction were found, except *Enterococcus faecalis* infections, which occurred significantly more frequently in pregnancies complicated preterm premature rupture of membranes.

cause of late preterm birth from 34 to 36 weeks of gestation [15,16]. However, the presence of infection is the main etiological factor of births from 30 to 32-week gestation. This is shown by histological examination of placenta and fetal membranes [1,2,17]. In such cases, the risk of fetal infection increases to 5-10%, which worsens the prognosis for its survival and subsequent health and psychomotor development.

Among the women spontaneously delivering prematurely, mostly identified the bacteria of relatively low virulence in microbiological testing. These include *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Gardnerella vaginalis*, *Peptostreptococcus spp.*, and *Bacteriodes*, *Neisseria gonorrhoea* and *Chlamydia trachomatis*. Microorganisms often caused genital tract infections of non-pregnant women, are rarely found in the pregnant uterus before rupture of membranes. However, organisms such as group B streptococci or *Escherichia coli*, occasionally occurring in non-pregnant women, often causing chorioamnionitis and fetal infection following the premature rupture of membranes. Most of the bacteria colonizing the uterine cavity associated with the induction of preterm labor is derived from the vagina. A significant inflammatory response manifested leucocyte infiltrates of membranes and an elevated concentration of pro inflammatory factors, including interleukin-6 in amniotic fluid is found in pregnant women with positive swabs from membranes [16,18].

Self analysis of vaginal bacterial flora of pregnant women showed a similar occurrence of natural flora and the lack of statistically significant growth of pathogenic bacteria cultured from swabs of the cervical canal of women giving birth prematurely, compared with which deliver at the time. In addition, there was no statistically significant increase in vaginal infections of β -hemolytic streptococci, *Enterobacteriaceae* group bacteria and *Candida* in women in preterm labor.

Regan in the "Vaginal Infections and Prematurity Study" showed that approximately 21% of women are colonized by *Streptococcus agalactiae*. The study demonstrated that carriage of group B streptococci in the genital tract is negatively correlated with bacterial vaginosis. Occurrence of β -hemolytic streptococcus is not

associated in a statistically significant way with preterm delivery, premature rupture of membranes, and intrauterine death, however, is correlated with preterm birth of low birth weight infant among pregnant massively colonized. Our findings confirmed this thesis. Pregnancy and childbirth in women moderately colonized has a similar way as in women with normal flora of the birth canal, with no significant statistical difference. It has also been a significant reduction in the number of preterm births and newborns with low and very low birth weight among women massively colonized, treated with an effective and guided antibiotic therapy [5]. Carey and Klebanoff [1] demonstrated that infection with *Escherichia coli* and *Klebsiella pneumoniae* contributes significantly to increase of preterm births. In our research has confirmed that infection with *Klebsiella spp.*, etiology was positively correlated with preterm birth and the occurrence of *Escherichia coli* in the cervical canal had no effect on pregnancy. According to Feikin, the colonization of the genital tract of more than 24 weeks of pregnancy by *Streptococcus agalactiae* is negatively correlated with the occurrence of lactobacilli and anaerobes. However, it may be an important risk factor for preterm delivery [17].

In prospective studies Kubota showed that streptococcal β -hemolytic colonization of the birth canal is not associated with a statistically significant increase in preterm birth and premature rupture of membranes. But in the case of coinfection with other organisms (especially *Gardnerella vaginalis* and anaerobic) and the reduction of lactobacilli, statistically significant increase in the occurrence of preterm birth and PROM was found [19,20].

Hillier confirmed this demonstrating that the presence of bacterial vaginosis in pregnancy in the second trimester is correlated with a higher risk of preterm delivery, infant of low birth weight below 2500g, irrespective of other risk factors diagnosed [3]. At the same time, no correlation between infection and the premature rupture of membranes was showing. Hay showed that the bacterial vaginosis in early pregnancy is associated with a higher percentage of late abortions between 16 and 24 weeks of gestation and preterm birth [11].

The relationship between preterm birth and genital infection was investigated by many authors. We found a significant correlation between premature delivery and pathogenic microorganisms with high virulence other non- β -hemolytic streptococci and bacteria from the group of *Enterobacteriaceae*. This applies mainly *Klebsiella spp.*, *Corynebacterium*, and *Pseudomonas aeruginosa*. According to Sherman isolation of microorganisms such as *Klebsiella spp.*, *Pseudomonas aeruginosa*, *Bacteroides spp.*, and *Gardnerella vaginalis* from the reproductive tract

was associated with an increase rate of premature birth [14]. It was also found a higher incidence of inflammation of the placenta in the histopathological examination of delivery below 37 weeks of gestation, especially when the etiologic agent were Gram - negative bacteria. In conclusion, the colonization of *Streptococcus agalactiae* or *Enterobacteriaceae* alone do not induce preterm labor and PROM. Only lowering the percentage of lactobacilli or coinfection with other microorganisms of high virulence increase the risk of premature contractions.

Another problem is the premature rupture of membranes in preterm pregnancy, which can be both a cause and a consequence of intrauterine infection. Infection within the blastocyst is the most difficult problems of modern perinatology. It is assumed that infection is accompanied by about 40% of all premature births. Therefore, in pregnant women with rupture of membranes, it is necessary to thorough and repeated bacteriological laboratory diagnostics, and allows you to monitor infection risk. Multicenter "Vaginal Infection and Prematurity Study demonstrated that carriage of β -hemolytic streptococci in the birth canal is not associated with premature rupture of membranes irrespective of the duration of pregnancy. Pregnancy and childbirth in women colonized only slightly was not statistically different from healthy pregnant women. However, severe, symptomatic GBS infection of the genital tract is associated with pPROM [5].

Because the vagina is colonized by a number of potentially pathogenic microorganisms, in theory, broad-spectrum antibiotics provide more benefits than narrow-spectrum drugs. However, a possible side effect of chemotherapeutic agents with a broad spectrum activity is the induction of anti-microbial resistance. Edwards et al demonstrated that antibiotic therapy in patients with pPROM is associated with an increased proportion of gram-negative bacteria resistant to ampicillin and causing sepsis in neonates [21,22].

Chance of a proper risk assessment of preterm delivery increases with a combination of methods for detection of its occurrence. Markers of preterm delivery in these secretions and body fluids such as blood, cervico-vaginal secretion and amniotic fluid are essential for optimal management in clinical practice. Exact knowledge of the bacterial flora colonizing the birth canal in different trimesters of pregnancy would allow for targeted and effective prevention of preterm birth. Only in those cases where there is suspicion that the cause of preterm labor is an intrauterine infection, the mother should receive antibiotics during pregnancy and childbirth. In other cases, the unjustified use of antibiotics can promote the selection of bacterial strains with increased virulence and induce drug resistance mechanisms [23].

CONCLUSIONS

1. The bacteria of low virulence, physiologically colonize the gastrointestinal tract, such as *Escherichia coli*, *Enterococcus faecalis*, or *Streptococcus agalactiae* is not an etiological factor of preterm labor and premature rupture of membranes.
2. Coinfection other microbes or bacteria with high virulence such as *Klebsiella* or *Pseudomonas* increase the risk of preterm labour.

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