

Evaluation of vaginal microbiota in women admitted to the hospital for premature labour

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Abstract. *Background and aim of the work:* The aim of this study was to evaluate the vaginal microbiota of women admitted to the hospital for premature labour and to compare the flora of those who responded to the tocolytic treatment with the flora of those who did not respond to the treatment and delivered prematurely. *Materials:* the hospital records of 245 women admitted to the division of Obstetrics and Gynaecology of 'Guglielmo da Saliceto' Hospital in Piacenza for premature labour, between 24 completed weeks and 36 weeks plus 6 days of pregnancy, were reviewed and the results of vaginal swabs collected on admission were evaluated. *Results:* a vaginal dysbiosis, with reduction or absence of lactobacilli and presence of pathogenic microbial species, was found in all women of our cohort admitted to the hospital for premature labour. Among them, 200 women (81,63%) responded to the tocolytic treatment with arrest of the contractions and could be eventually discharged, while 45 women (18,36%) did not respond to the treatment and delivered prematurely (before 37 completed weeks). The four microbial species most commonly isolated in the vaginal flora were: *Ureaplasma urealyticum*, *Streptococcus agalactiae*, *Candida albicans* and *Gardnerella vaginalis*. When the characteristics of the vaginal flora of the two groups were compared, a more severe dysbiosis, with absence of lactobacilli and evidence of more than one pathogenic microbial species, was found in 18% of women who responded to the tocolysis and in 71,4% of women who did not respond. The difference was statistically significant ($p < 0,05$, two-tailed test). *Conclusions:* vaginal dysbiosis was diagnosed in all women admitted to the hospital for premature labour. A more severe dysbiosis, with complete absence of lactobacilli and presence of two or more pathogenic microbial species, was prevalent in the majority of women who showed with a poor response to the tocolytic treatment compared to women with minor degrees of alteration of the vaginal flora. (www.actabiomedica.it)

Key words: Vaginal flora, Dysbiosis, Premature labour, Tocolytic treatment

Background and aim of the work

Reproductive health in women requires an adequate microbial homeostasis in both reproductive and digestive tracts. Premature delivery is the main cause of perinatal mortality and morbidity with long term consequences for the newborn. Its prevention is one of the main challenges of modern obstetrics. Premature labour is often preceded by rupture of the membranes,

urinary tract infection or endometritis (1). Pregnant women with vaginal dysbiosis, either anaerobic vaginosis or aerobic vaginitis, have a 1,5 times higher risk to go into premature labour compared to women with a normal vaginal flora (2-4).

Vaginal flora represents a balanced ecosystem of microbial species which, depending on their metabolic characteristics, can be divided in aerobic and anaerobic (5). Lactobacilli are the dominant microbial species in

the vagina of women in good health. They represent the natural defence against pathogens and carry out this task through production of organic acids, hydrogen peroxide, bacteriocins with antimicrobial activity and modulation of the host immune response (6). The interface between the mucosa of the female reproductive tract and the external environment represents the first defence line against the invasion of pathogenic bacteria and sexually transmitted diseases.

During pregnancy maternal body undergoes important physiologic changes to support foetal and placental growth with a weight gain which, in women with a normal body mass index (between 18 and 25), ranges between 9 and 12 kilograms. Intestinal dysbiosis may interfere with this physiologic adaptation by influencing the absorption of nutrients introduced with the food (7,8).

Premature labour often occurs in the presence of vaginal dysbiosis with concomitant presence of pathogenic microbial species: the onset of uterine contractions is assumed to be due to an ascending infection which, initially asymptomatic, is rarely identified and treated in the early stages (9,10). Soon after birth the foetal intestine is colonised by microorganisms which may vary according to birth modalities (caesarean section or vaginal delivery), type of feeding (breast or artificial) and whether antibiotics have been administered during labour in patients who have tested positive for streptococcus at the time of hospital booking. Complete maturation of intestinal microbiota in the child occurs between age two and five. Antibiotic treatments may alter the composition of vaginal flora, particularly ampicillin, cephalosporins and vancomycin, that have a stronger effect on lactobacilli compared to other antibiotics such as metronidazole, sulphonamides, chinolones and tetracyclines (11).

The use of probiotics to preserve a normal vaginal microbiota may be useful in principle as a preventive measure against premature labour caused by an ascending vaginal infection. Probiotics have the capacity to colonise the vaginal flora and correct the dysbiosis. Their administration to the mother in pregnancy and during breast feeding can strengthen the immunoprotective potential of maternal milk and reduce the incidence of allergic reactions and atopic eczema in the neonate (12,13).

Epidemiologic studies have shown that an abnormal bacterial environment of the lower genital tract may increase the susceptibility to HIV virus

infection (14), the occurrence of pelvic inflammatory disease (15) and miscarriages (16,17).

Methods

This was a retrospective observational study therefore by Italian law (n 72, 26/3/2012) Ethics Committee approval was not sought. Collection of vaginal swabs, for which women gave their verbal consent, is a standard procedure in our hospital as part of the investigations of women admitted for premature labour. The results of culture and sensitivity serve as a guideline in the choice of the antibiotic treatment to select.

The composition of vaginal microbiota was evaluated in a cohort of 245 women admitted to the 'Guglielmo da Saliceto' Hospital in Piacenza, a hospital of the Italian National Health Service, with a diagnosis of premature labour in order to compare the difference between the flora of those who responded to the tocolytic treatment and the one of those who did not respond and delivered prematurely.

A diagnosis of premature labour was formulated on the basis of the following:

- 1) gestational age between 24 completed weeks and 36 weeks and 6 days.
- 2) presence of regular uterine contractions felt by the patients and recorded by cardiotocography
- 3) cervical length of less than 25 mm on vaginal ultrasound

Patients with ruptured membranes on admission were excluded from the study.

The management protocol of premature labour included the following: a complete blood count, urea and electrolytes, ESR and C reactive protein. High vaginal swabs were collected in all patients and sent to the microbiology laboratory for culture and sensitivity.

If the diagnosis of premature labour was confirmed the patients were initially hydrated with an intravenous infusion of crystalloids (Ringer's Acetate Solution, 1000 ml). Steroids were administered by intramuscular injection to prevent neonatal respiratory distress syndrome (betamethasone 12 mg, two doses

given at 24 hours interval). If an elevated white cell count or increased C reactive protein were present or the patient was pyrexial, intravenous broad spectrum antibiotics were commenced. If contractions persisted in spite of intravenous hydration then Atosiban, an oxytocin antagonist, was commenced by intravenous infusion according to the standard protocol. The broad spectrum antibiotics regimen started initially on admission was modified if culture and sensitivity of the vaginal swabs results suggested to do so.

Results

The pathogenic microbial species identified in the vaginal flora are summarised in Figure 1.

Marital status, ethnicity, age and occupation of patients were not taken into account in our evaluation. All patients declared they were not smoking in pregnancy, although a small percentage (34%) admitted they were smoking before getting pregnant and decided to quit when the pregnancy test became positive. The mean age was 32 years, but stratification by age groups did not show any significant link between age and premature labour. In the cohort of 245 women

we evaluated 81.63% (n = 200) responded to the tocolytic treatment and carried on with the pregnancy uneventfully until term. In 18.36% (n= 45) the tocolytic treatment was ineffective and a premature delivery occurred. Vaginal swabs showed evidence of severe dysbiosis with two or more pathogenic bacteria and absence of lactobacilli in only 18% of women who responded to the treatment and in the majority, 71,4%, of women who did not respond. The difference between the two groups was statistically significant ($p < 0.05$, two tailed test). The microbial species most frequently isolated in the vaginal flora were *Ureaplasma urealyticum*, *Streptococcus agalactiae*, *Candida Albicans* and *Gardnerella vaginalis*. The most frequent combinations of pathogens are shown in Table 1.

Discussion

The term dysbiosis identifies any variation in the normal bacterial flora. Diet composition has a very important role in maintaining a normal intestinal flora if the right amounts of prebiotics and probiotics are present (18). Prebiotics promote the growth of intestinal bacteria which are useful for the host. Probiotics

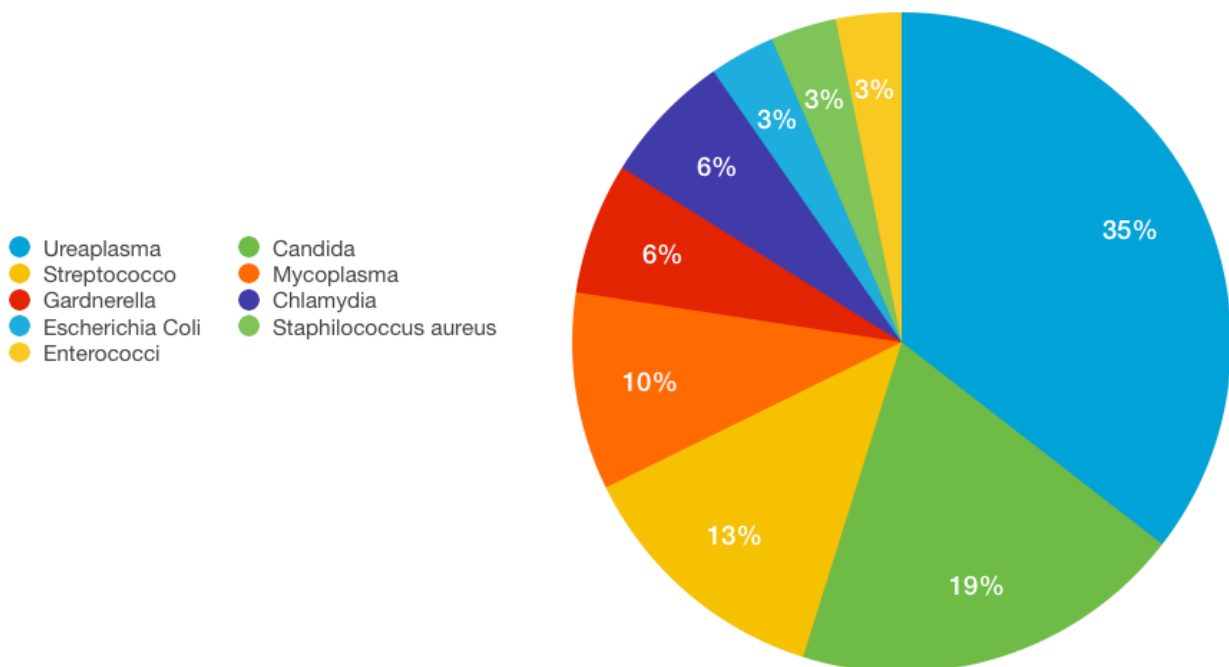


Figure 1. Pie diagram of prevalence of microbial species isolated in vaginal flora

Table 1. The most frequent combination of pathogens found in vaginal flora

Microbial associations found in vaginal dysbiosis		
Candida Albicans	Streptococcus	
Candida Albicans	Ureaplasma	
Candida Albicans	Chlamydia	Ureaplasma
Candida Albicans	Mycoplasma	Ureaplasma
Candida Cruzei	Streptococcus	Ureaplasma
Candida Glabrata	Ureaplasma	
Chlamydia	Gardnerella	
Enterococci	Ureaplasma	
Escherchia Coli	Ureaplasma	
Gardnerella	Ureaplasma	
Mycoplasma	Streptococcus	
Mycoplasma	Ureaplasma	
Staphilococcus	Ureaplasma	
Streptococcus	Ureaplasma	

are microorganisms that have a positive effect on the health of the host. The composition of intestinal microbiota is important for a normal function of the digestive and reproductive systems. The last segment of the large bowel plays an important role as a reservoir of microorganisms capable of colonising the vagina (19). Intestinal flora can also influence sex hormones metabolism and the incidence of endometriosis, a disease caused by endometrial tissue that grows outside the uterine cavity in three different forms: ovarian, peritoneal and infiltrating (20,21). Prebiotics introduced with the diet during pregnancy increase the chances of the birth of a healthy baby who is also more resistant to infections (9). A diet too rich in fat can have the opposite effect altering maternal milk composition, which is paramount for the development of a normal microbiota in the neonate (22)

An enteric-mammary circle has been identified which peaks towards the end of the pregnancy and during breast feeding. (23,24).

In pregnancy antimicrobial peptides have a key immunologic role at the level of the amniotic membranes and placenta, acting as a barrier against ascending infections (25). The main species of lactobacilli present in the vaginal flora are *Lactobacillus Acidophilus*, *L. Crispatus*, *L. Gasseri*, *L. Jensenii* and *L. Iners*. An alteration of vaginal flora may produce a bacterial vaginosis characterised by a prevalence of anaerobic species such as *Gardnerella*, *Mobiluncus*, *Bacteroides*, *Prevotella* and *Peptostreptococcus*, or an aerobic vaginitis, with prevalence of *Streptococcus*, *Staphylococcus*, *Escherichia Coli*. Bacterial vaginosis is a frequent finding in women in the reproductive age. An indicator of its presence is a vaginal discharge with a typical 'fishy' odour. This is due to amines produced during bacterial metabolism (26) The clinical diagnosis of bacterial vaginosis is based on the presence of a vaginal discharge which the patient describes as 'smelly' and on cultural and microscopic evaluation of the discharge. The correct diagnosis may be difficult unless vaginal swabs are collected. Aerobic vaginitis is characterised by a stronger local inflammatory reaction and immune response: inflammatory cytokines and interleukins 1, 6 and 8 produced with chorioamnionitis and these are deemed to be the trigger stimulus for the onset of premature labour (27, 28).

An adequate level of glycogen and a low vaginal pH value due to lactic acid production are important conditions to ensure survival of lactobacilli.

The ascending infection from the lower genital tract finds no valid opposition if lactobacilli are absent (29). When the inflammatory cascade mechanism is activated (30) in response to pathogenic bacteria, cytokines and endotoxins, arachidonic acid and prostaglandins are produced which may stimulate uterine contractions while enzymes like metalloproteinases have the capacity to modify and weaken cervical collagen and foetal membranes (31, 32).

Conclusions

An alteration of normal vaginal microbiota was diagnosed in all women of our cohort admitted to the hospital for premature labour.

A more severe dysbiosis, with marked reduction or total absence of lactobacilli and presence of more than

one pathogenic microbial species, was present in the majority of women who showed no response to the standard tocolytic treatment.

We assume this may be due to a more severe flogistic reaction with production of higher levels of inflammatory cytokines which ultimately exert a stronger stimulus on uterine contractility which the medical treatment is unable to counteract.

Early diagnosis of bacterial vaginosis and aerobic vaginitis in pregnancy may help to identify women at risk of developing premature labour who could benefit from measures aimed at recreating a normal vaginal flora. A large population study will be necessary to clarify whether correction of vaginal dysbiosis in asymptomatic pregnant women will have a significant impact on the onset of premature labour.

Conflicts of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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