

# Prevalence, Spectrum and Antibiotic Susceptibility of Bacterial and Candida Colonization between the 21st and 33rd Week of Gestation in Women with PPROM – 5 Years' Experience in 1 Perinatal Center

Bakterien- und Candida-Besiedlung zwischen der 21. und 33. Schwangerschaftswoche bei Frauen mit vorzeitigem Blasensprung (PPROM): Verbreitung, Spektrum und Resistenz – 5 Jahre Erfahrungen in einem Perinatalzentrum

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## Key words

- preterm premature rupture of membrane
- antibiotic susceptibility
- bacterial and Candida colonization
- prevalence

## Schlüsselworter

- vorzeitiger Blasensprung
- Antibiotikaresistenz
- Bacteria- und Candida-Kolonisation
- Pravalenz

received 31.8.2012  
revised 15.12.2012  
accepted 16.12.2012

## Bibliography

DOI <http://dx.doi.org/10.1055/s-0032-1328132>  
Geburtsh Frauenheilk 2013; 73: 59–62 © Georg Thieme Verlag KG Stuttgart · New York · ISSN 0016-5751

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## Abstract

**Objective:** The aim of this study was to evaluate the prevalence, spectrum and antibiotic susceptibility of bacterial and Candida colonization of the vagina between the 21st and the 33rd week of gestation in women who had preterm premature rupture of membranes (PPROM).

**Study design:** High vaginal swabs from 245 subjects with PPROM were analyzed in a retrospective cohort study using cultivation-dependent methods. Patients were additionally divided into two groups: women with PPROM between the 21st and 27th week of gestation (group A) and women with PPROM between the 28th and 33rd week of gestation (group B). A subgroup analysis comparing the two groups was done.

**Results:** The prevalence of pathological bacterial colonization was similar in both study groups (40.8 vs. 41.4%;  $p > 0.05$ ), however, a difference in antibiotic susceptibility was noted, which did not reach statistical significance (resistance to ampicillin 71.4 vs. 52.5%; cefuroxime 9.5 vs. 11.7%; gentamicin 28.6 vs. 16.4%; ciprofloxacin 5.0 vs. 5.4%). In group A there was a statistically significant lower rate of Candida colonization (11.1 vs. 24.3%;  $p = 0.04$ ).

**Conclusion:** In patients with early PPROM, the rate of Candida colonization (group A) is lower and there are indications of a difference in antibiotic susceptibility of the colonizing bacteria depending on gestational age. Larger study groups are required to confirm these preliminary results.

## Introduction

In Europe the rate of preterm births is about 5 to 9% of all births. However in the USA the preterm birth rate is even higher (12–13%) and the rates are increasing, despite a better knowledge of the

## Zusammenfassung

**Einleitung:** Ziel dieser Studie ist die Untersuchung von Pravalenz, Spektrum und Antibiotikaresistenz der Bakterien- und Candida-Kolonisation der Scheide nach vorzeitigem Blasensprung in der 21. bis zum Abschluss der 34. Schwangerschaftswoche.

**Material und Methode:** Hohe vaginale Abstriche von 245 Schwangeren mit vorzeitigem Blasensprung wurden retrospektiv mit Kultivierung analysiert. Eine Subgruppe von Schwangeren in der 21. bis zum Abschluss der 27. Schwangerschaftswoche (Gruppe A) wurde mit Schwangeren in der 28. bis zum Abschluss der 33. Schwangerschaftswoche verglichen.

**Ergebnisse:** Die Pravalenz bakterieller Kolonisation war in beiden Gruppen in vergleichbarer Hufigkeit vorhanden (40,8 vs. 41,4%,  $p > 0,05$ ). Es zeigte sich jedoch eine unterschiedliche Antibiotikaresistenz in beiden Gruppen; der Unterschied war statistisch nicht signifikant (Resistenz gegen: Ampicillin 71,4 vs. 52,5%; Cefuroxim 9,5 vs. 11,7%; Gentamicin 28,6 vs. 16,4%; Ciprofloxacin 5,0 vs. 5,4%). In Gruppe A zeigte sich eine statistisch signifikant niedrigere Candida-Kolonisation (11,1 vs. 24,3%;  $p = 0,04$ ).

**Schlussfolgerung:** Schwangere mit sehr fruhem vorzeitigem Blasensprung haben eine niedrigere Candida-Kolonisation und moglicherweise eine andere Antibiotikaresistenz abhangig vom Schwangerschaftsalter. Groere Studien sind notwendig, um diese vorlaufigen Ergebnisse zu bestatigen.

risk factors involved [1]. This increase is partly due to rising numbers of medically indicated preterm births (for maternal or fetal indications) and to the increase in assisted reproductive technologies [1,27]. Children born preterm have a higher risk for major disabilities, such as cerebral palsy

and respiratory morbidity, and are more likely to develop behavioral and educational difficulties [2,3]. The risk of subsequent morbidities and long-term problems is increased in children born at a younger gestation age [2,3,26]. 25% to 30% of preterm births are associated with preterm premature rupture of membranes (PPROM). Thus, optimal management of patients with PPRM is a major priority in the prevention of preterm birth. Causes of and risk factors for PPRM are multifactorial and include uterine ischemia or hemorrhage, tobacco exposure, however the most important causes are infection and inflammation [1]. Di Giulio et al. demonstrated that microbial invasion of the amniotic cavity affected half of all patients with PPRM (detected by molecular methods), although invasion was frequently not detected using culture-based methods, which suggests that molecular techniques (e.g. PCR) may be more sensitive for the detection of amniotic infections [4–6]. The same study group found PPRM to be more frequently associated with *Candida* spp. infection than was previously known [4]. The pathophysiology of infection causing PPRM is considered to be due to the production of prostaglandins and matrix-degrading enzymes via microbial endotoxins and pro-inflammatory cytokines (e.g. IL-8, IL1 $\beta$ , TNF $\alpha$ ) which, in turn, are released after the binding of microorganisms to pattern-recognition receptors (e.g. toll-like receptors) [1]. Prostaglandins play a major role in stimulating uterine contractions, while degradation of the extracellular matrix in fetal membranes is implicated in PPRM [1,7]. However, infection caused by ascending microorganisms may also occur secondary to PPRM [8,9]. Intra-uterine infection has been demonstrated in up to 60% of cases [8,9]. More recent data suggest that some microbes invade the amniotic cavity from the bloodstream after dissemination from remote sites, e.g. from the gastrointestinal tract [4].

The appropriate therapy for women with PPRM is still unclear. The ORACLE Children Study found that the administration of antibiotics to patients with PPRM was neither beneficial nor harmful for children's health at a follow-up of 7 years [10–12]. In women who had spontaneous labor with no preterm rupture of membranes, there was some evidence of fetal harm due to the administration of antibiotics. This highlights the importance of ascertaining that the diagnosis of PPRM is correct before prescribing antibiotics [11,12].

In patients with PPRM, the administration of antibiotics appears to offer some benefit, especially at an earlier gestational age (<32 weeks), and is associated with significant benefits in short-term outcomes (prolongation of pregnancy, prevention of chorioamnionitis and neonatal infection). Thus, antibiotic therapy is still the standard of care [13,14]. However, if antibiotics are prescribed, it is still unclear which should be the antibiotic of choice. Penicillins and macrolide antibiotics can be administered alone or in combination, parenterally or orally. The administration of amoxicillin plus clavulanic acid to pregnant mothers was found to be associated with a significantly higher risk of necrotizing enterocolitis in infants after delivery, and it was recommended not to prescribe this combination to pregnant women [15,16]. Thus, more information on the prevalence, spectrum and antibiotic sensitivity of bacterial and *Candida* colonization is important to determine the optimal treatment strategies for patients with PPRM. The aim of our study was therefore to provide data on the prevalence and antibiotic sensitivity of bacterial and *Candida* colonization in patients with PPRM to improve the knowledge base for decisions on the use of specific antibiotics. We also carried out a subgroup analysis comparing prevalence, sensitivity and colonization in early PPRM (21st – 27th week of

gestation) with that in late PPRM (28th – 34th week of gestation).

## Methods



### Study population

All samples were collected at the perinatal center of Johann Wolfgang Goethe University Frankfurt, Germany, between June 2006 and May 2011. A retrospective cohort study was done in patients with PPRM who met the following inclusion criteria: (i) gestational age between 21 and 34 weeks, and (ii) high vaginal swabs taken on admission to hospital for microbiological diagnosis prior to receiving antibiotic treatment. A subgroup analysis was done to compare findings in the 21st to 27th week of gestation (group A) with those in the 28th to 33rd week of gestation (group B).

### Study protocol

Membrane rupture was diagnosed by insulin-like growth factor binding protein-1 (IGFBP-1; Actim<sup>®</sup> PROM). After the diagnosis of PPRM, a high vaginal swab was taken for microbiological culture of aerobic and anaerobic bacteria and fungi. Microbiological culture techniques were performed in accordance with standard laboratory techniques [17]. Bacterial susceptibility testing was done according to the recommendations of the Clinical and Laboratory Standards Institute, Wayne, PA, USA [18]. All procedures were performed by the routine diagnostic laboratories of the Institute of Medical Microbiology and Infection Control of the University Hospital of Frankfurt (Germany). These laboratories are certified in accordance with DIN EN ISO/IEC 17025 and 15189 standards (laboratory identification code: D-ML-013102-01 and D-PL-13102-01-00).

### Statistical analysis

All results are shown as mean values  $\pm$  standard deviation. Wilcoxon signed rank test and Fisher's exact test were used for statistical analysis. Analysis was done using SPSS Statistics 17.0 software (Scientific Packages for the Social Sciences, Inc., Chicago, IL, USA). A value of  $p < 0.05$  for a two-tailed test was considered statistically significant.

## Results



Cervical swabs obtained from 245 subjects with PROM were analyzed. **Table 1** presents the baseline characteristics of the 245 enrolled patients. Aerobic bacteria were identified with culture testing (**Table 2**).

The cervical prevalence of bacteria which colonize normal vaginal flora (*Escherichia [E.] coli*, *Streptococcus agalactiae*, *Klebsiella* spp. etc.) and are potentially involved in amniotic infections was similar in both study groups (40.8 vs. 41.4%;  $p > 0.05$ ), however a difference in the susceptibility to antibiotics was noted, although this difference was not statistically significant (overall resistance to ampicillin 71.4 vs. 52.5%; resistance to cefuroxime 9.5 vs. 11.7%; resistance to gentamicin 28.6 vs. 16.4%; resistance to ciprofloxacin 5.0 vs. 5.4%). **Table 3** shows the individual susceptibility to antibiotics for the majority of pathogenic bacteria. Group A had a significantly lower rate of *Candida* colonization (11.1 vs. 24.3%;  $p = 0.04$ ) and the difference was statistically significant.

**Table 1** Characteristics of the study population (n = 245; n.s. = not significant).

Characteristic	Group A 21st to 27th week of gestation (n = 56)	Group B 28th to 33rd week of gestation (n = 189)	p-value
Maternal age (years)	32.6 ± 4.3	33.0 ± 5.4	n.s.
Nulliparity	62.3% (33/53)	67.1% (110/164)	n.s.
Candida colonization	11.1% (6/54)	24.3% (37/152)	0.04
Bacterial colonization	40.8% (21/54)	41.4% (63/152)	n.s.
Normal vaginal bacteria	55.6% (30/54)	46.1% (70/152)	n.s.
Overall resistance			
▶ ampicillin	71.4% (15/21)	52.5% (32/61)	n.s.
▶ cefuroxime	9.5% (2/35)	11.7% (7/60)	n.s.
▶ gentamicin	28.6% (6/35)	16.4% (10/61)	n.s.
▶ ciprofloxacin	5.0% (1/20)	5.4% (3/56)	n.s.

**Table 2** Bacteria identified on culture (n = 206; n.s. = not significant).

Bacteria species	Group A 21st to 27th week of gestation (n = 54)	Group B 28th to 33rd week of gestation (n = 152)	p (Fisher's exact test)
<i>E. coli</i>	5.6% (3/54)	13.2% (20/152)	n.s.
<i>Enterococcus faecalis</i>	0% (0/54)	0.7% (1/152)	n.s.
<i>Staphylococcus aureus</i>	5.6% (3/54)	2.6% (4/152)	n.s.
Normal vaginal species	55.6% (30/54)	46.1% (70/152)	n.s.
<i>Streptococcus agalactiae</i>	5.6% (3/54)	5.3% (8/152)	n.s.
<i>Streptococcus dysgalactiae</i>	0% (0/54)	0.7% (1/152)	n.s.
<i>Klebsiella pneumoniae</i>	5.6% (3/54)	3.3% (5/152)	n.s.
<i>Klebsiella oxytoca</i>	3.7% (2/54)	0% (0/152)	n.s.
<i>Salmonella enteritidis</i>	0% (0/54)	0.7% (1/152)	n.s.
<i>Enterobacter cloacae</i>	0% (0/54)	2.6% (4/152)	n.s.
<i>Pseudomonas aeruginosa</i>	0% (0/54)	0.7% (1/152)	n.s.
<i>Proteus mirabilis</i>	0% (0/54)	1.3% (2/152)	n.s.
<i>Proteus vulgaris</i>	0% (0/54)	0.7% (1/152)	n.s.
<i>Citrobacter koseri</i>	0% (0/54)	0.7% (1/152)	n.s.
<i>Acinetobacter lwoffii</i>	0% (0/54)	1.3% (2/152)	n.s.
No growth	18,5% (10/54)	20.4% (31/152)	n.s.

**Table 3** Resistance of *E. coli*, *Streptococcus agalactiae* and *Klebsiella* spp. (n = 245; n.s. = not significant).

Characteristic	Group A 21st to 27th week of gestation (n = 56)	Group B 28th to 33rd week of gestation (n = 189)	p-value
<i>E. coli</i> resistance			
▶ ampicillin	66.7% (8/12)	55.0% (22/40)	n.s.
▶ cefuroxime	16.7% (2/12)	2.5% (1/40)	n.s.
▶ gentamicin	25.0% (3/12)	5.0% (2/40)	n.s.
▶ ciprofloxacin	8.3% (1/12)	7.5% (3/40)	n.s.
<i>Streptococcus agalactiae</i> resistance			
▶ ampicillin	0% (0/2)	0% (0/6)	n.s.
▶ cefuroxime	0% (0/2)	0% (0/6)	n.s.
▶ gentamicin	100% (2/2)	100% (6/6)	n.s.
▶ ciprofloxacin	0% (0/2)	0% (0/6)	n.s.
<i>Klebsiella</i> spp. resistance			
▶ ampicillin	100% (4/4)	100% (1/1)	n.s.
▶ cefuroxime	0% (0/4)	0% (0/1)	n.s.
▶ gentamicin	0% (0/4)	0% (0/1)	n.s.
▶ ciprofloxacin	0% (0/4)	0% (0/1)	n.s.

## Conclusion

The study showed that the prevalence of bacteria colonizing the normal vaginal flora and potentially involved in amniotic infection in PPRM was around 41% for both subgroups. These results correspond to previously published data [4, 19, 20]. It is interest-

ing to note that 10 and 71%, respectively, of detected bacterial species were resistant to the commonly used broad-spectrum antibiotics cefuroxime and ampicillin. The major pathogenic bacteria showed a varied susceptibility to antibiotics (Table 3). Different antibiotic susceptibilities were noted for group A (21st to 27th week of gestation) compared to group B (28th to 33rd week

of gestation). Group A showed a higher resistance to broad-spectrum ampicillin (71.4 vs. 52.5%) while resistance to cefuroxime was higher in group B, although at a markedly lower level (9.5 vs. 11.7%). These findings are in accordance with data showing increasing numbers of antibiotic-resistant *E. coli* infections among preterm infants. Ampicillin-resistant *E. coli* infections would seem to be linked to prolonged antenatal administration of antibiotics in cases with PPRM and appear to preferentially affect preterm infants [21]. Furthermore, the intrapartum administration of ampicillin has been shown to be an independent risk factor for ampicillin-resistant *E. coli* early-onset sepsis and also increases *E. coli* late-onset sepsis [21]. Thus, there is a need for further and larger studies evaluating the bacterial spectrum and antibiotic sensitivity in patients with PPRM, with a special focus on certain subgroups of patients, as the data suggests that different treatment approaches should be used for patients in the 21st to 27th week of gestation and for patients in the 28th to 33rd week of gestation. We found some aerobic bacteria, but most vaginal bacteria are anaerobic, especially in women with bacterial vaginosis which commonly affects around 20% of the study population [22].

Special emphasis should be laid on treating *Candida* colonization, especially later in pregnancy, as our subgroup B had a significantly higher rate of *Candida* colonization (11.1 vs. 24.3%;  $p=0.04$ ). The finding that *Candida* colonization is significantly higher later on in pregnancy is well known [23]; two studies noted the impact of vaginal *Candida* colonization on preterm birth but it is still not fully understood and has not been investigated in prospective studies [24, 25]. As mentioned above, infection with *Candida* spp. was found to be more frequently associated with PPRM than was previously thought [4] and that effective therapy should be administered to prevent preterm birth. Future studies should include outcome measures (e.g. intra-amniotic inflammation at presentation, histopathologic inflammation of the placenta and chorioamniotic membranes at delivery) as well as pregnancy and perinatal outcomes.

## Clinical Practice

Around 41% of women with PPRM have pathological bacterial contamination of the vagina resistant to the most commonly administered broad-spectrum antibiotics ampicillin and cefuroxime.

## Conflict of Interest

None.

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