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CLINICAL ARTICLE

# Antibiotic susceptibility pattern of genital tract bacteria in pregnant women with preterm premature rupture of membranes in a resource-limited setting

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ABSTRACT

*Objective:* To identify microbes prevalent in the genital tract of pregnant women with preterm premature rupture of membranes (PPROM) and to assess the susceptibility of the microbial isolates to a range of antibiotics to determine appropriate antibiotics for treating cases of PPROM in resource-limited settings. *Methods:* A prospective cross-sectional study was undertaken involving women with (n = 105) and without (n = 105) a confirmed diagnosis of PPROM admitted to Nnamdi Azikiwe University Teaching Hospital, southeast Nigeria, between January 1, 2011, and April 30, 2013. Endocervical swabs were collected from all participants and examined microbiologically. Antibiotic sensitivity testing was performed using Kirby–Bauer disk diffusion. *Results: Streptococcus* spp., *Staphylococcus aureus*, and *Escherichia coli* were significantly more prevalent among women with PPROM than among those without PPROM ($P < 0.01$). Among the antibiotics considered safe to use during pregnancy, the bacteria were most sensitive to ampicillin-sulbactam, cefixime, cefuroxime, and erythromycin. *Conclusion:* For the first 48 hours, women with PPROM should receive an intravenous dose combining ampicillin-sulbactam, cefixime, cefuroxime, or erythromycin with metronidazole followed by oral administration of the chosen antibiotic combination to complete a 7-day course.


## 1. Introduction

Preterm premature rupture of membranes (PPROM) refers to the rupture of the fetal membranes prior to the onset of labor at less than 37 weeks of pregnancy [1,2]. It occurs in 3% of pregnancies and is responsible for approximately one-third of all preterm births [3]. Despite the many advances in perinatal care, PPROM remains a potentially serious complication with significant risks of maternal and fetal morbidity [3–6]; studies have shown that there is a strong association between PPROM and infection [7].

In contrast to preterm labor, the administration of antibiotics in cases of PPROM forms part of the current standard of care, principally because there is strong evidence indicating that antibiotics protract pregnancy and decrease short-term neonatal morbidity [8–10]. The administration of antibiotics eradicates intrauterine infection, decreases the inflammatory response, and prevents invasion by ascending microbial agents [8]. To institute appropriate prophylaxis or therapy, amniotic fluid cultures and endocervical and high vaginal swabs should be taken to determine the antimicrobial susceptibility pattern of the isolated organisms.

Surprisingly, in resource-poor settings, broad-spectrum antibiotics are often prescribed without microbiological studies [11,12]. Consequently, there is the strong likelihood of antibiotic abuse, a situation that poses serious public health problems in many low-income countries.

A range of broad-spectrum antibiotic regimens have been examined, and there is no evidence to recommend one regimen over another. In the USA, the most common regimen is that which was performed in the National Institute of Child Health and Human Development (NICH) trial on PPROM, which recommended an initial 48 hours of intravenous therapy with ampicillin and erythromycin, followed by 5 days of oral therapy with amoxicillin and an enteric-coated erythromycin base [10,13,14]. Several studies have attempted to determine

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other protocols for antibiotic therapy after PPROM, but have not resulted in any definitive recommendations, particularly in low-income countries [8–10].

The evaluation of antibiotic susceptibility patterns of genital tract microbial agents in women with PPROM continues to be a daunting task in resource-poor settings. The parenteral formulation of erythromycin as recommended by the NICH study is not readily available in Nigeria. Owing to the prevailing antibiotic resistance in low-income countries, treatment with ampicillin may not be appropriate in cases of PPROM. The aims of the present study were to identify the microbes prevalent in the genital tract of pregnant women with and without a confirmed diagnosis of PPROM, and to determine the antibiotic susceptibility pattern of microbes isolated from women with PPROM to be able to recommend an antibiotic treatment regimen or protocol in line with the NICH study of PPROM that can be safely administered in cases of PPROM in healthcare institutions with inadequate or no laboratory facilities.

## 2. Materials and methods

A prospective cross-sectional study was undertaken involving pregnant women admitted to Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, southeast Nigeria, between January 1, 2011 and April 30, 2013. NAUTH is a tertiary hospital that serves as a referral center for many cases of PPROM. The PPROM group comprised women between 28 and 37 weeks of pregnancy presenting to the Labor and Delivery Suite of the hospital with a confirmed diagnosis of PPROM. The non-PPROM comparison group comprised pregnant women without rupture of membranes that were invited to enroll in the study while attending prenatal clinics at NAUTH. The women in the PPROM and non-PPROM groups were matched for age (±2 years), parity, and gestational age (±2 weeks). Women were excluded from the PPROM group when they had had PPROM more than 24 hours prior to presentation, had previous digital examination before presentation, received antibiotic treatment within 7 days of presentation, PPROM with temperature up to 38 °C, or active vaginal bleeding. Ethical approval was obtained from the Institutional Review Board of NAUTH and written informed consent was obtained from all the participants.

Endocervical swabs were taken from all recruited women. The study was single blinded: specimens were coded and numbered consecutively in such a way that the microbiologist analyzing specimens did not know whether they were from the PPROM or the non-PPROM group.

All women were evaluated for rupture of membranes with the aid of a detailed history, a physical examination, and a sterile speculum examination. A diagnosis of membrane rupture was made at the initial examination using standard clinical assessment criteria if two of the following three clinical signs were present: a visual pooling of fluid in the posterior fornix, a positive nitrazine test, or microscopic evidence of ferning [11,15]. The main outcome measures were the detection of microorganisms and the antibiotic susceptibility of the microorganisms.

All the samples were analyzed in the laboratory by a senior medical laboratory scientist. The samples from both groups were inoculated onto plates of dried chocolate agar, blood agar, MacConkey agar, and Sabouraud dextrose agar. All plates were incubated at 37 °C for 24–48 hours. A few drops of saline were added to each swab after inoculation and then placed on a microscope slide and examined under the microscope.

Samples that developed cultures of microorganism(s) were subjected to an antibiotic susceptibility test using a modified Kirby–Bauer disk diffusion method [16] on chocolate agar plates using Oxoid multidiscs with standard antibiotic concentrations [16]. Zone sizes were measured and interpreted according to the Clinical Laboratory Standards Institute [16].

Based on a previous study of microbial agents among pregnant women in hospital that reported a prevalence rate of 17% for *Gardnerella vaginalis* [17], a minimum sample size of 91 participants per group was calculated to be required to achieve a power of 85% to detect a difference in prevalence between 3.5% (non-PPROM) and 17.0% using $\chi^2$ (two-sided) with continuity correction and a significance level of $P < 0.05$. Power analysis and sample size calculations were performed using PASS version 12 (NCSS, Kaysville, UT, USA) and Epi Info version 3.5.1 (Centers for Disease Control and Prevention, Atlanta, GA, USA). To account for a potential 10% loss to follow-up, 105 women were enrolled in each group. Data were analyzed with Epi Info version 3.5.1 and Stata version 10 (StataCorp, College Station, TX, USA) using $\chi^2$ tests and Fisher exact tests as appropriate to determine whether there was any significant difference in the prevalence of the bacterial species isolated in the PPROM and the non-PPROM groups.

## 3. Results

In total, 210 women participated in the study (Fig. 1). The demographic characteristics of the participants are shown in Table 1. Both groups were apparently homogenous ($P > 0.05$). The mean age of the women in the PPROM group was 30.7 ± 5.4 years and the mean age of the non-PPROM group was 30.4 ± 5.4 years. Most women in the study population had a parity of 0–2. The mean gestational age of the fetuses in the PPROM and non-PPROM groups were 31.5 ± 1.7 weeks and 31.4 ± 1.8 weeks, respectively.

Bacteria were recovered from 83 (79.1%) of the samples taken from women with PPROM ($P < 0.001$) (Table 2). *Streptococcus* spp. were the bacteria isolated most frequently in the PPROM group; these bacteria were identified significantly more frequently in the PPROM group than the non-PPROM group (Table 2).

In the PPROM group, ampicillin-sulbactam was the most effective antibiotic: 81 (97.6%) of the bacterial isolates cultured from the 83 specimens were sensitive to ampicillin-sulbactam (Table 3). Amoxicillin, ampicillin-cloxacillin, and co-trimoxazole all showed low levels of effectiveness in this group: only 21 (25.3%), 25 (30.1%), and 25 (30.1%) isolates, respectively, were sensitive to these antibiotics. Few *Proteus mirabilis* isolates were sensitive to gentamicin or ceftriaxone (Table 3). The sensitivity of isolates from the non-PPROM group is shown in Table 4.

## 4. Discussion

In the present study, the following microbial agents were detected and were significantly associated with PPROM: *Streptococcus* spp., *Staphylococcus aureus*, and *Escherichia coli*. Whether these bacteria are direct causes of PPROM or are simply surrogate markers for another as yet unidentified pathogenic process remains to be determined. Thus, in contrast to preterm labor, there is strong evidence to support administration of antibiotics in cases of PPROM.

The rationale for prophylactic treatment of PPROM with antibiotics is that infection appears to be both a cause and consequence of PPROM, and can lead to premature delivery [18]. The goal of antibiotic therapy is to reduce the frequency of maternal and fetal infection and to prolong the latency period. In the present study, among the 13 antibacterial agents tested, ampicillin-sulbactam, streptomycin, gentamicin, cefixime, cefuroxime, ciprofloxacin, ceftriaxone, erythromycin, and co-amoxiclav were the most effective. However, studies have shown that treatment with streptomycin during pregnancy is not safe, and co-amoxiclav has been found to cause neonatal necrotizing enterocolitis [9,10]. Additionally, erythromycin is not available in parenteral formulation in Nigeria and is not active against anaerobes, Group B streptococcus, or many of the organisms associated with bacterial vaginosis [9].

Gentamicin was effective against 72 (86.7%) of the bacterial isolates, which supports the findings by Aboyeji et al. [7] that gentamicin is a broad-spectrum antibacterial agent. However, gentamicin is unavailable in oral formulation and has the ability to potentiate nephropathy and ototoxicity. Gentamicin should therefore only be used in places where alternative and more effective drugs are unavailable or where

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