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Original Research Article

Microbial Isolate Among Women with Premature Rupture of Foetal Membranes and Pregnancy Outcome in National Hospital Abuja.

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ABSTRACT

Background: Premature rupture of foetal membranes is a significant contributor to perinatal morbidity and mortality, and in the tropics where there is lack of facilities for neonatal intensive care unit, this poses a significant therapeutic dilemma in current obstetrics practice. **Aim**: To determine the profile of microorganisms, their antimicrobial sensitivity pattern, gestational age at delivery, mode of delivery, cases of neonatal sepsis and cases of endometritis. **Methodology**: A prospective study was conducted among pregnant women presenting with premature rupture of foetal membranes at National Hospital Abuja. The Participants were recruited consecutively and a structured interviewer questionnaire was completed. Endocervical and high vaginal swab were collected to isolate microorganisms and their sensitivity pattern was investigated, the subjects were followed up till delivery and outcome of pregnancy was noted. **Results**: A total of 101 cases of PROM were analysed, pathogens were isolated in 20.9% of cases. The common organisms isolated are *Candida albicans* (8.9%), *Staphylococcus aureus* (5.0%) and *Escherichia coli* (3.0%). Most of the organisms were sensitive to Ceftriaxone, Amoxyclav and Imepemen. Vaginal delivery was the mode of delivery in 56.4% and 51.5% had preterm delivery. Neonatal sepsis was seen in 3% of the new born and no cases of maternal endometritis were recorded. **Conclusion**: It has been shown from this study that some pathogens are associated with PROM. The study also found that Ceftriaxone and Imepenem were the antibiotics with highest sensitivity. Therefore, Prophylactic use of antibiotic should be based on demonstrated microbial pattern and their sensitivity. Further research is needed to explore the association between PROM and *Candida albicans* as the commonest organism seen.

Keywords: Microbial Isolate, Sensitivity, PROM, Microorganism.

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INTRODUCTION

Intact foetal membranes with normal amniotic fluid volume are necessary for normal foetal growth and development. The membranes serve as a barrier that separates the sterile foetal environment from the bacteria colonised vagina.[1] Premature rupture of membranes (PROM) is defined as rupture of the foetal membranes prior to onset of labour.[1,2] Rupture of membranes beyond 37th week is Term PROM and when it occurs before 37 completed weeks is Preterm PROM (PPROM). Rupture of membrane for more than 24hours before delivery is prolonged rupture of membrane.[2,3]

Premature rupture of membranes complicates 10% of pregnancies. [4-6] Preterm PROM affect 2% of pregnancies and is associated with 30 – 40% of preterm delivery and is the leading cause of prematurity with its associated consequences.[4,7] It affects 120,000 - 150,000 pregnancies in the United States each year. [6,7] It has a tremendous socio-economic impact in the society.[1]

The aetiopathogenesis of PROM is complex and multifactorial.[1] A number of mechanism have been proposed these include intrinsic membrane weakness, mechanical stress, and ascending infection which is the greatest risk factor.[1,5,6] There is evidence demonstrating an association between ascending infection from the lower genital tract and PROM.[8] It has been demonstrated that bacterial product such as proteases and phospholipases decrease the strength, elasticity of the membrane and stimulate the release of prostaglandins leading to preterm contractions.[1,6] Infection also causes host immune response releasing proinflammatory cytokines and mediators causing weakening of the membranes by disrupting its extracellular matrix and releasing metalloproteinase, hence increasing the risk of PROM.[1] Microbial organism implicated in PROM are polymicrobial and include *Trichomonas vaginalis*, *Chlamydia trachomatis, Neisseria gonorrhea*, *Group B streptococci* and those causing *Bacteria vaginosis*, women infected with such organisms have an increased risk of PPROM.[1] Organisms isolated in some studies in Nigeria include: *staphylococcus auresu, Gardnerela vaginalis, Candida, Strep. Pyogens* among others. [5,9]

The diagnosis of PROM is made from patient history which has a sensitivity of 90%, and is confirmed by a sterile speculum examination to evaluate for pooling of liquor in the posterior fornix of the vagina or trickling of liquor from the cervix, some simple test may be performed.[1,3,6,8] The interval between rupture of membranes and onset of labour is called latent period of leaking, which is the key factor for determining maternal and foetal outcomes.[6]

PROM is a significant risk factor for both preterm birth, maternal and early neonatal sepsis. Studies has shown that prompt laboratory screening for infection and early institution of broad-spectrum antibiotic significantly improved neonatal outcome, by preventing infections, prolonging the latency period and increasing gestational age at delivery, thereby reducing the incidence of maternal and neonatal morbidity and mortality. [6,8,10-12]

Although several studies have been conducted on microbial isolate and sensitivity pattern in women with PROM in Nigeria where different microbial flora and their sensitivity pattern were noted, but no study was found from Nigeria in the course of literature search, on microbial isolate before and 24 hours after delivery, their sensitivity pattern and pregnancy outcome. It is based on this background that this study was designed to identify microbial isolate before and 24 hours after delivery, their antibiotic sensitivity pattern, gestational age at delivery, mode of delivery, neonatal outcome, cases of neonatal sepsis and cases of maternal endometritis. Findings from this study will help improve the clinical management of our patient.

The aim of this study was **to** determine the profile of microorganisms among women with PROM between gestational age of 28 weeks to 42 weeks at National Hospital Abuja.

Objectives

1. To determine the microorganism isolates associated with PROM and the antimicrobial susceptibility pattern of microorganism(s) isolated
2. To determine microbiological isolate before and 24hours after delivery
3. To determine the gestational age at delivery, mode of delivery and neonatal outcome
4. To determine if there are cases of neonatal sepsis
5. To determine if there are cases of endometritis

METHODS

The study was a prospective study conducted from 1st October 2017 to 31st August 2018, in the Department of Obstetrics and Gynaecology National Hospital Abuja among pregnant women with PROM between 28 to 42 weeks of gestation, confirmed by sterile speculum examination with absence of labour who consented to participate. Those excluded include PROM on antibiotic and who had previous digital examination before presentation.

The minimum sample size was determined by using the formula [13]: $n= z^2pq/d^2$

Where:

n = minimum sample size required

z =z – score corresponding to desire confidence level at 95% (z=1.96)

p= estimated proportion of variable of interest in the population = 6.8% [14] (i e 0.068)

d= tolerable margin of error or precision usually expressed as a decimal, 0.05 for a 5% margin of error

q = 1 - p (proportion of population without the characteristic)

For this study,

p= 0.068

d= 0.05

z= 1.96

q=1- 0.068 = 0.932

Sample size $n = (1.96)^2 \times 0.068 \times 0.932 / 0.0025$

n= 97.4 approximately 97 subjects.

Adjustment for expected response rate: to accommodate for attrition, (with the anticipation of a 90% response rate), attrition rate = 100% - 90% = 10%, therefore attrition rate is 10%= 0.10

The estimated adjusted sample size, $n=n/1 – R= n/1 – 0.10$, Thus $n_s = 97/0.9=107.8$ approximately 108 subjects.

Ethical approval was obtained from the National Hospital Abuja Health research and ethic committee with assigned number NHA/EC/007/2017.

Eligible women that presented to the labour ward or Gynae emergency with PROM between 28 weeks to 42 weeks' gestation were consecutively recruited into the study until required sample size was obtained. At recruitment informed consent was obtained from each participant. A detailed history was obtained, participants were physically examined and a sterile

speculum examination was conducted to confirm PROM (evidence by pooling of liquor in the posterior fornix of the vagina or trickling from the cervical os). Under aseptic condition a separate sterile swab stick was used to collect from both the endocervical secretion and high vagina, each swab was placed in the swab container which was properly labelled with identification number for each participant that is similar with the labelling on data collection form to avoid cross contamination and was immediately send to the laboratory for analysis by the laboratory scientist already pre informed.

A structured interviewer questionnaire was completed for all the participants to obtain information on their bio data and other information relevant for the study. The participants were followed up till delivery. Mode of delivery (vaginal or caesarean section), the gestational age at delivery and neonatal outcome were noted. Twenty-four hours after delivery a repeat endocervical swab for microbiological study was taken to determine organism before and after delivery(participant were already counselled and pre informed at recruitment), and the participants were assessed for clinical feature of endometritis by temperature charts monitoring for fever(T-38 degree Celsius and above), abdominal examination for uterine tenderness and abnormal foul-smelling lochia during their hospital stay, educated before discharge on how to recognize symptoms at home, and re assessed at the post-natal clinic (first visit , at 2 weeks post-partum). Before their scheduled postpartum visit, the mothers were regularly contacted via mobile phone by the principal investigator to hear from them about their state of health. The neonate after delivery were assessed together with the neonatologist for features of neonatal sepsis using full blood count and blood culture.

Specimen Processing

At the laboratory the registered sample were inoculated on various media which include blood agar, MacConkey agar, Chocolate agar, and Thayer Martins media. These were incubated in air at 37^{o}C for 24 – 48 hours. A wet preparation from the specimen was then made by adding few drops of saline to each swab and then put on a slide mount under microscope to examine for *the* presence of *trichomonas vaginalis trophozoites, motile bacteria, Candida, epithelial cells* and pus cells. After 24-48hrs of inoculation the media plates were observed for any growth and the morphology of the bacteria described, a direct gram smear is made after identifying the morphology and stained using crystal violate, loguls iodine, acetone and safranin, the slides are then viewed under microscope to identify the organisms, these were further subjected to various biochemical tests in order to identify the specific bacteria isolated, using standard procedure and algorithm,

The significant pathogen(s) identified were further evaluated for antimicrobial susceptibility testing using the kirby-Bauer method, these were then interpreted using the interpretation chart and the organisms were reported sensitive, intermediate and resistant. The antibiotic used are: Ceftriaxone, Erythromycin, Amoxiclav, Cotrimoxazole, Imipenem, Ciprofloxacin, Gentamicin and Ampicillin.

The data obtained was imputed into an SPSS computer statistical software version 23 and analysed using same software. Categorical variables were presented using proportion and percentage.

RESULTS

During the study period from 1st October 2017 to 31st August 2018, 108 patients with premature rupture of membranes were recruited. Of the 108, seven patients were lost to follow-up and hence excluded from the study. The analysis was based on 101 patients with PROM.

Table 1. Socio-Demographic Characteristics of the Study

	Frequency (n=101)	**Percentage (100%)**
Age		
20 – 24	7	6.9
25 – 29	29	28.7
30 – 34	44	43.6
35 – 39	17	16.8
40 – 44	3	3.0
≥ 45	1	1.0
Occupation		
House wife	57	56.4
Student	4	4.0
Civil Servant	16	15.8
Business Woman	24	23.8
Educational status		
Primary	1	1.0
Secondary	36	35.6
Tertiary	64	63.4
Parity		
Primigravida	40	39.6
Para 1	23	22.8
Para 2	28	27.7
Para 3	6	5.9
Para 4	4	4.0

Table 2. Microbial Isolate

Micro organism	HVS N (10)	ECS Before Delivery N (8)	ECS After Delivery N (3)
Candida albicans	7 (6.9)	1(1.0)	1(1.0)
Staphylococcus aureus	2(2.0)	3(3.0)	0(0)
Escherichia coli	0(0)	2(2.0)	1(1.0)
Enterococcus spp	0(0)	1(1.0)	0(0)
Trichomonas Vaginalis	1(1.0)	0(0)	0(0)
Streptococcus viridians	0(0)	0(0)	1(1.0)
Providentia spp	0(0)	1(1.0)	0(0)

Table 3. Microbial Sensitivity Pattern

Drugs	Number Sensitive and Percentage					
	Staph aureus n =5 (%)	E. coli n =3 (%)	Enterococcus n = 1 (%)	Strept spp n = 1 (%)	Providencia n = 1 (%)	Total Number 11(100%)
Ceftriaxone	5(100)	1 (33.3)	1(100)	1(100)	1(100)	9(81.8)
Erythromycin.	3(60)	0(0)	0(0)	0(0)	0(0)	3(27.3)
Amoxyclav	3(60)	3(100)	1(100)	1(100)	0(0)	8(72.7)
Cotrimaxozole	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Imepenem	3(60)	3(100)	1(100)	1(100)	1(100)	9(81.8)
Ciprofloxacin	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Gentamicin	0(0)	3(100)	0(0)	0(0)	0(0)	3(27.3)
Ampicillin	0(0)	0(0)	1(100)	0(0)	0(0)	1(9.1)

Table 1 shows the socio-demographic characteristics of the study. The age range of the women was 20 to 45 years with mean age of 31.32± 4.77. majority are house wives with tertiary level of education. Primigravida account for 39.6%.

A total of 21 organisms were isolated from both HVS and ECS. *Candida albicans* was isolated in 9 (8.9%) of cases, *Staphylococcus aureus* in 5 (5.0%) and *Escherichia coli* in 3 (3.0%), *Enterococcus spp, Trichomonas vaginalis, streptococcus viridans and providentia spp 1(1%)* as shown in Table 2.

Eight antibiotics were tested against cultured organisms. The drugs with highest sensitivity were Ceftriaxone (81.8%), Imepenem (81.8%) and Amoxyclav (72.7%). Those with least sensitivity are Erythromycin (27.3%), Gentamicin (27.3%) and Ampicillin (9.1%). All organisms are resistant to Ciprofloxacin and Co-trimoxazole. *Staphylococcus aureus* was 100% sensitive to Ceftriaxone and 60% to Erythromycin, Amoxyclav and Imepenem. *Escherichia coli* was 100% sensitive to Amoxyclav, Gentamicin and Imepenem. While *Enterococcus spp, Streptococcus viridans* and *Provedentia* are 100% sensitive to Ceftriaxone, Amoxyclav and Imepenem as shown in Table 3 below.

E. coli (Escherichia coli), Strept spp (Streptococcus spp), Staph aureus (Staphylococcus aureus)

Majority of the patient had preterm delivery 54(56.4%). High percentage occurred at estimated Gestational age of 28-33 weeks. 91.1% of the cases presented with < 12-hours onset of PROM (Table 4) and majority (55.4%) had latency period of > 24 hours. Fifty-two of the patients had spontaneous vaginal delivery accounting for 51.5%, while 49(48.5%) delivered by caesarean section.

Of the 101neonate delivered,56.4% were delivered preterm and 52.5% had low birth weight. 33.6% had first minute Apgar score less than 7 and 51.55 were admitted into NICU and 6 neonatal deaths

occurred. Only 3 neonate developed neonatal sepsis (Table 4).

Table 4. Gestational Age at Delivery / Neonatal Outcome

Variable	Frequency	Percentage (%)
Gestational Age (Weeks)		
28 – 33	40	39.6
34 – 36	14	16.8
37 – 42	44	43.6
Duration of PROM		
< 12 hours	92	91.1
>12 hours	9	8.9
NEONATAL OUTCOME		
Prematurity	57	56.4
Low birthweight	53	52.5
Apgar score <7 in 1minute	34	33.6
NICU Admission	52	51.5
Death	6	5.9
Neonatal sepsis	3	3.0

DISCUSSION

In this study 21 organisms were isolated from both High vaginal and Endocervical swab accounting for 20.9% (9.9% from HVS and 11.0% from ECS) which is lower compared to findings of 44.4% and 48.3% from Nigeria,[9] and Togo [14] respectively.

The organisms isolated are *Candida albicans (8.9%), Staphylococcus aureus (5.0%), Escherichia coli (3%), Trichomonas vaginalis (1%), Enterococcus spp (1%), Streptococcus viridans (1%) and Providencia spp (1%)* in decreasing order. This study is similar to findings of Gahwagi et al,[16] were *Candida albican* was the most frequent organism isolated followed by Gardnerella vaginalis which was not isolated in this study.This is comparable to the study findings of Karat et al,[17,] Aboyeji et al,[9] Gichuhi et al,[18] Bharathi et al[19] and Zeng et al,[20] although Aboyeji et al found *Gardneralla vaginalis* as the most common organism which was not isolated in this study, and Karat et al found *Staph aureus* as the commonest organism followed by *E coli* and Candida *albican*. Gichihi et al found *E. Coli* as commonest organism also *Enterococcus spp, Trichomonas vaginalis and Streptococcus viridian* were isolated as found in this study. It is not yet clear whether these organisms isolated are direct causes of PROM or they are simply incidental findings, however all the isolated organisms were earlier documented as possible risk factors. [5,9,21]

Candida albicans was the most common organism isolated in the present study. The association between the Candidiasis and rupture of membrane is still unclear, however, there is evidence of release of inflammation Cytokines during Candida infestation which may cause membrane rapture.[22]

Staphylococcus aureus was isolated in this study as seen in other studies. [5,9,17] Recent evidence implicated *Staph aureus* as an emerging cause of chorioamnionitis and premature rapture of membrane which are associated with preterm birth and neonatal disease.[23] According to the literature *Staph aureus* induced the release of pro inflammatory Cytokines which promotes neutrophils release of MMP that directly degrade membrane component.[23]

Escherichia coli was found in 3% of the cases and has been indicated in previous study to be capable of penetrating intact foetal membranes causing intra amniotic infection and subsequently rapture of membrane.[5] Other organisms that are implicated with PROM such as *Bactreriodes spp*, *Ureaplasma urealyticum*, *Chlamydia* among orders were not isolated due to lack of specific culture for them.

Of the 11.0% organisms isolated from endocervical swab, 8% were from ECS before delivery (at presentation) and 3% from ECS 24-hours after delivery as presented in Table 2 above. The 3 organisms isolated 24 hours after delivery are *E coli*, *Candida albicans* and *Streptococcus viridans*. Among the three organisms isolated above, one patient has a positive culture for *E coli* which was identical to the one isolated before delivery in her ECS, this could be due to the resistance of the organism to the prophylactic antibiotic(erythromycin) prescribed on before delivery. Although this assumption cannot be confirmed because the serotype of the organism was not done. The remaining two patients had sterile culture in their ECS before delivery. The presence of these organisms (*Candida albicans* and *Streptococcus viridans*) twenty-four hours after they delivered in their ECS could be due to contamination during vaginal birth.

The goal of antibiotic therapy is to reduce the frequency of maternal and foetal infection and to prolong the latency period.[21] Among the eight antibacterial agents tested, Ceftriaxone (81.8%) and Imepenem (81.8%) shows highest sensitivity followed by Amoxyclav (72.7%). While Erythromycin (27.3%), Gentamicin (27.3%) and Ampicillin (9.1%) were least sensitive. All these drugs are safe in pregnancy, however Amoxyclav has been found to be associated with Necrotizing enterocollitis so is not recommended in PROM.[11] Ciprofloxacin and Co-trimoxazole shows 100% resistance to the organisms isolated. This finding is comparable to that of Eleje et al[21] which found them to be effective with Ampicillin-sulbactam as the most effective among them. However, Ampicillin-sulbactam was not among the drugs tested in this study. Also, Adewumi et al[24] found ceftriaxone, amoxiclav, cefuroxime and ciprofloxacin to have highest sensitivity in their study, but ciprofloxacin was shown in this study to be resistant to all the organisms isolated and the drug is not recommended to use in pregnancy.[24]

All the organisms isolated from this study shows100% sensitivity to Ceftriaxone except *E coli* (33.3%). Also, Erythromycin shows 100% resistance to all the organisms except *Staph aureus* that was 60% sensitive. This is important because Erythromycin and Ceftriaxone are the common antibiotic used in our practice.

In the present study 56.4% of the patients delivered preterm with highest percentage of 39.6% occurring at gestational age between 28 – 33 weeks, this is comparable to findings of Isaac et al (30-32 weeks).[26] And most of the patients presented < 12-hour onset of PROM and had latency period of >24-hours. Spontaneous vaginal delivery was the mode of delivery in 51.5% of the patients. This was similar to findings from various studies, [25,26,27] because PROM alone is not a contraindication to vaginal delivery. Those that had caesarean section have other added obstetric indications.

Total of 101 neonates were delivered during the study period, 51.5% of them were admitted into neonatal intensive care unit due to various indication notably prematurity, low birth weight and birth asphyxia. Only 3%of the neonate developed neonatal sepsis this is similar to 2.9% finding of Shivaraju et al[27] but lower than that of Isaac et al (10%).[26] The study also has no cases of endometritis during the puerperium which is similar to the findings of Salou et al[14] this could be due to the prophylactic antibiotic given to the patients.

CONCLUSSION

Microbial organisms were isolated in 20.9% of the patients, the common organisms are *Candida albicans, staphylococcus aureus and Escherichia coli.* Ceftriaxone, Imepenem and Amoxyclav are the most sensitive antibiotics. Therefore, Prophylactic use of antibiotic should be based on demonstrated microbial pattern and their sensitivity, also periodic review of these findings is necessary to look for emergence of antibiotic resistance and abuse. There is also need to further explore the association of candida with PROM as the commonest organism isolated in the study.

Limitations

Some microorganism like *Chlamydia tracomatis* and *Mycoplasma* spp could not be isolated due to lack of facility for their identification.

Recommendation

In light of the above, the study would recommend the following;

1. Ceftriaxone is recommended to be used in combination with Erythromycin as prophylaxis for PROM.
2. Routine endocervical swab should be taken in patient with PROM.
3. Further research is needed to explore the association of candida with PROM and other risk factors.

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QUESTIONNAIRE

MICROBIAL ISOLATE AMONG WOMEN WITH PREMATUER RUPTURE OF FOETAL MEMBRANES (PROM) AND PREGNANCY OUTCOME.

Dear Respondent,
This questionnaire on the above subject is for academic purpose, and also towards improving the management of pregnancies. All information obtained will be kept confidential.

A. **RESPONDENT'S SOCIO-DEMOGRAPHIC PROFILE**

1. Serial No……..
2. Hospital No……
3. Age…….
4. Occupation of patient……..
5. Occupation of husband…….
6. Educational Status of patient……
7. Educational status of husband……..
8. Address……..
9. Tribe……
10. Religion……..
11. Gravidity…….
12. Parity…….
13. LMP……..
14. EDD……

B. INDEX PREGNANCY:

15. Gestational Age……
16. Duration of rupture of membranes…
17. Any problem associated with the pregnancy e.g. vaginal bleeding, vaginal discharge, fever, etc…… …… ……
18. Were you given any antibiotic in the last 7 days?
    Yes [ ] NO [ ]

C. OUTCOME

19. Gestational age at delivery……
20. Mode of delivery
    a. vaginal delivery
    b. caesarean section
21. Any symptoms and signs suggestive of clinical infection?
    a. Maternal fever (temp. > 38oc) { }
    b. Raised maternal pulse (>100bpm) { }
    c. Abdominal tenderness { }
    d. Foul smelling PV discharge { }
    e. None of the above
22. foetal outcome
    a. Birth weight…..
    b. Apgar score….
    c. Neonatal sepsis. Yes { }, No { }
    d. Admission into NICU. Yes { }, No { }
23. maternal endometritis after delivery?
24. If yes specify…….
25. Endocervical swab (before and after delivery) result……
26. High vaginal swab result……

Thank you.