THE MICROBIAL PATTERN ASSOCIATED WITH PRETERM PREMATURE RUPTURE OF MEMBRANES AS SEEN AT MUHIMBILI NATIONAL HOSPITAL

By

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A Dissertation submitted in partial fulfillment of the requirement for the award of the degree of Master of Medicine (Obstetrics and Gynaecology) of the Muhimbili University of Health and Allied Sciences

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I, **Dr Furaha August** declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other University or academic institution for a similar or any other academic award.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences, a dissertation titled: *The Microbial Pattern associated with Premature Rupture of Membranes as seen at Muhimbili National Hospital*, in partial fulfillment of the requirements for the Degree of Master of Medicine – Obstetrics and Gynaecology

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DEDICATION

This dissertation is dedicated to my wife Abella and my young daughter Imani, who were forced to miss my attention temporarily because of this work. TO my mother Prof Siriel Massawe and my father Dr Augustine Massawe for their care and upbringing and made sure I got education and reach this stage.

ABSTRACT

Preterm Premature Rupture of Membranes (PPROM) affects about 3% of pregnancies worldwide. It is associated with significant maternal, fetal and neonatal complications. Previous studies in Muhimbili National Hospital had shown only the prevalence of PPROM. In MNH there is no data to show the microbial pattern of PPROM which is important in the management of patients with PPROM. This study sought to address this gap.

Objectives: To describe microbial pattern associated with preterm premature rupture of membranes and their antimicrobial sensitivity pattern.

Study Population: Pregnant women admitted in the labour ward with PPROM and PROM and not in labor.

Study design; A descriptive cross -sectional study

Methods: A structured questionnaire was used to collect Information on socio-economic status and obstetric characteristics. Endocervical and high vaginal swabs were collected to isolate micro organisms present in the genital tract. Cervical smears were gram stained and evaluated microscopically. Sensitivity patterns were also investigated

Results: A total of 110 cases of PPROM and 220 of PROM were analyzed Pathogens were isolated in 50/110(45.5%) in patients with PPROM compared to only 11.5%

isolated from patients with PROM. The common microbes isolated in PPROM were *E.Coli* (30%), *Klebsiella* (24%), *Candiasis* (10%), *Staphylococcus aureus* (4%) *Trichomonas vaginalis* (14%), *Neisseria gonorrheae* and *Staphylococcus aureus* were not isolated in patients with PROM. This difference was statistically significant. Bacterial Vaginosis was isolated in the PPROM group was 36% while in the PROM group it was 23%.Ceftriaxone and Gentamicin were found to have appropriate susceptibility pattern. Common isolates were found to be resistant to Ampicilin, Chloramphenicol.

Conclusion; It has been shown from this study that some pathogens were associated with PPROM and PROM and that Ceftriaxone and Gentamicin were the antibiotics with appropriate sensitivity. It is emphasized that women with PPROM should have endocervical swabs taken so as to know the anti microbial sensitivity.

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Abbreviations

MNH	-	Muhimbili National Hospital
PPROM	-	Preterm Premature Rupture of Membranes
PROM	-	Premature Rupture of Membranes
ROM	-	Rupture of Membranes
MUHAS	-	Muhimbili University of Health and Allied Sciences
PMTCT	-	Prevention of Mother to Child Transmission
HIV	-	Human Immunodeficiency Virus

INTRODUCTION

The terminology pertaining to premature rupture of membranes (PROM) can be confusing, so understanding the subtle distinctions can be valuable. PROM is rupture of the membranes (ROM) prior to the onset of labor. PROM is used appropriately when referring to a patient who has a gestation of 37 weeks and beyond, has presented with spontaneous rupture of the membranes (SROM), and is not in labour. Preterm Premature Rupture of Membranes (PPROM) is ROM before the onset of labour in a patient with less than 37 weeks of gestation. Midtrimester PROM is premature rupture of membranes between 16 and 26 weeks. Currently it is clinically relevant to differentiate preterm PROM into "previable PROM [which occurs before the limit of viability (less than 23 weeks)], "preterm PROM remote from term" (from viability to about 32 weeks) and preterm PROM near term (from 32 to 36 weeks of gestation). Lastly Prolonged Preterm Premature Rupture Of Membranes refers to PPROM without delivery for an interval greater than 24 hours. [1-3]

Eighty five percent of neonatal morbidity and mortality has been associated with prematurity. Preterm premature rupture of the membranes (PPROM) is associated with 30 – 40% of preterm deliveries and is the leading identifiable cause of prematurity. The most common associated risk factors for PPROM are smoking, previous preterm delivery, and vaginal bleeding at any time during the index pregnancy.

PPROM may also occur in patients with an incompetent cervix, which can result from previous genital tract surgery (such as surgically induced abortion or a D & C or laceration[1].

Incidence of PROM is approximately 10% of pregnancies. Patients with PROM may present with complaint of leaking fluid, vaginal discharge, and or sometimes vaginal bleeding, but they are not in labour.[1, 2, 4]

Pathogenesis of PPROM.

The etiology of PPROM is multifactorial. Various mechanisms have been proposed, including mechanical, as well as infectious or inflammatory process. Choriodecidual infection or inflammation appears to play an important role in etiology of PPROM especially at early gestational age[4]. Decreased membrane collagen content has been demonstrated in the setting of preterm PROM and with increasing gestational age. In support of this, increases in amniotic fluid Matrix Metalloproteinases as well as tissue inhibitors of matrix Metalloproteinases have been identified among women with PPROM[5].

PPROM occurs with increased frequency in women who smoke or who have multiple gestation, polyhydramnios, or ante partum hemorrhage. Some women with PPROM also appear to have inherent deficiencies in collagen synthesis, which may predispose to weakening of the membranes[5].

Of greatest interest in recent years has been the confirmation that PPROM is associated with lower and upper genital tract infection; there are 3 major lines of supporting evidence.

(1) Many of the bacteria that inhabit the lower genital tract can produce phospholipase A, an enzyme that can trigger the arachidonic acid cascade that leads to the synthesis of prostaglandins. These same bacteria also are able to produce a variety of proteolytic enzymes that can degrade the collagen matrix of the chorioamniotic membranes[1].

(2) Compared to women with uncomplicated gestation, those with PPROM are more likely to have lower genital tract infections (such as group ß-streptococcal colonization or Bacterial Vaginosis).

(3) Compared to women with preterm labour and intact membranes, women with PPROM are more likely to have clinical and sub clinical chorioamnionitis and inflammatory cytokines in the amniotic fluid.

Each of the above may be associated with preterm PROM through membrane stretch or degradation, local inflammation or a weakening of maternal resistance to ascending bacterial colonization[5,6].

Management of PROM

Management of PPROM usually presents a dilemma to the physician. It may be dictated by the presence of overt intrauterine infection, advanced labour or fetal compromise. When such factors are not present especially with preterm PROM, other interventions may have significant impact on maternal and infant morbidity. An accurate assessment of gestational age and knowledge of the maternal, fetal and neonatal risks are essential in the management [2-3]. Accurate diagnosis should me made and preservation of sterility is paramount importance

For a woman who has PPROM at 32 – 36 weeks of gestation, expeditious delivery is recommended. This is because prolongation of pregnancy is associated with increased chorioamnionitis, and prolonged maternal hospitalization. It is recommended that fetal pulmonary maturity be assessed by vaginal pool if available, and that amniocentesis by a skilled clinician be considered should there be inadequate vaginal fluid for pulmonary maturity evaluation.

For a woman between 23- 31 weeks of gestation, conservative management with the use of antenatal corticosteroids, tocolytics and antibiotics are used. Here inpatient observation of the patient is advocated[2,3].

The use of antibiotics in PPROM has been associated with a delay in delivery and reduction in major markers of neonatal morbidity such as respiratory distress syndrome, necrotizing enterocolitis. Similarly the use of antibiotics has been shown to reduce

maternal infectious morbidity namely chorioamnionitis and endometritis. Therefore the goal of adjunctive antibiotic therapy is to treat or to prevent ascending decidual infection in order to prolong pregnancy and offer opportunity for reduced neonatal infectious and gestational age-dependant morbidity[7]. In the Maternal Fetal Medicine Networks Study Ampicilin and erythromycin were used, compared to placebo. Patients who received antibiotics remained undelivered after 7 days and significant reduction in respiratory distress syndrome, neonatal death and intraventricular hemorrhage[8].

Kenyon et al performed a meta-analysis of antibiotics (amoxyl clavunate) for PPROM and it was shown that women were less likely to deliver within 48 hours, deliver within 7 days, and did not develop chorioamnionitis. But there was increased risk of neonatal necrotizing enterocolitis[9].

Widespread use of antibiotics in PPROM has shown some resistant organisms to Ampicilin and erythromycin[10]. The use of microbial cultures of the cervical vaginal secretions have been recommended especially in isolating Group β-Streptococci which is associated with adverse neonatal mortality. This is especially used in patients who had previous PPROM because the use of antibiotics in subsequent pregnancy has shown to prevent PPROM and Preterm Birth. Patient with previous BV should also be treated with Metronidazole for seven days [7,9 - 13]

In our set up the management of PPROM is usually by giving of antibiotics for seven days and corticosteroids for the patient whose gestation age is less than 34 weeks. The antibiotics used here are Ampicilin and Metronidazole.

LITERATURE REVIEW

Despite advances in perinatal care, preterm premature rupture of the membranes continue to present an important obstetrical challenge. At term, 8 to 10 percent of pregnant women present with premature rupture of the membranes; these women are at increased risk for intrauterine infection when the interval between the membrane rupture and delivery is prolonged[1, 2].

Preterm premature rupture of the membranes occurs in approximately 1 percent of all pregnancies and is associated with 30 to 40 percent of preterm deliveries. It is thus the leading identifiable cause of preterm delivery (before 37 completed weeks' gestation) and its complications, including respiratory distress syndrome, neonatal infection, and intraventricular hemorrhage.

Sub clinical intrauterine infection has been implicated as a major etiological factor in the pathogenesis and subsequent maternal and neonatal morbidity associated with PPROM. The frequency of positive cultures obtained by transabdominal amniocentesis at the time of presentation with PPROM in the absence of labor is 25 – 40%. The majority of amniotic fluid infection in the setting of PPROM does not produce the signs and symptoms traditionally used as diagnostic criteria for clinical chorioamnionitis[14].

In a study of PPROM in pregnancies at 16 – 26 weeks gestational age, the prevalence of positive amniotic fluid cultures was 30% In preterm labor patient with intact

membranes, positive bacterial cultures were present in amniotic fluid specimen obtained by amniocentesis before PPROM approximately 10% of the time[15]

Several studies demonstrate an association between colonization of the genital tract by group B-*streptococci*, *Chlamydia trachomatis, Neisseria gonorrhea*, and the microorganisms that cause bacteria vaginosis (vaginal anaerobes, *Gardnerella vaginalis*, *Mobiluncus* species, and genital *mycoplasma*) and an increased risk of preterm premature rupture of the membranes furthermore, in some studies treatment of infected women with antibiotics decreased the rate of preterm premature rupture of the membranes. Intrauterine infection may predispose women to rupture of the fetal membranes through any of several mechanisms, each of which induces degradation of extracellular matrix. Several organisms that are commonly present in the vaginal flora, including group B-streptococci, *Staphylococcus aureus, Trichomonas vaginalis*, and the microorganisms that cause bacterial vaginosis, secrete proteases that can degrade collagen and weaken the fetal membranes[1, 2]. In study done in Dakar University hospital the incidence of preterm birth was 15 per 1000 childbirth. The three principal etiologies were preeclampsia, preterm premature rupture of membranes and infections[16].

In a study done in Dominican Republic on microbial isolates associated with PPROM, patients with preterm PROM, microbial invasion of the amniotic cavity by

Streptococcus agalactiae, F. nucleatum or F. influenza was associated with high frequency of adverse maternal and neonatal outcomes and neonatal death [17]

Cervicovaginal infections were significantly associated with PROM ($p<0.001$), PPROM ($p<0.001$) and preterm delivery (p0.001), but not with intrauterine death, this was found in a study done in Italy. In the same study the microorganisms most frequently found were: yeasts 44%, *Ureaplasma urealiticum* (29%); group *B streptococci* (15%) and Bacteria vaginosis (11%). Cervicovaginal cultures were positive in 84.6% of PPROM, 55.0% of PROM, 50.8% of preterm deliveries, 43.8% of mid-trimester miscarriages, and 31.4% of intrauterine deaths and in 33.5% of controls [18].

In a study done in South Korea on PPROM, intra-amniotic inflammation, regardless of culture result, was present in 42% of patients with preterm premature rupture of the membranes and that it was a risk factor for impending preterm delivery and adverse outcome.[19] Aboyeji et al in a study to investigate the bacteriology of PROM in Nigeria found that *Gardnerella vaginalis, Candida, Staphylococcus aureus, Strep. pyogenes* were common microorganism associated with PROM [20].

Asymptomatic group B-streptococci colonization of the genital tract has been associated with PPROM and preterm birth. Treatment to eradicate the colonization is beneficial in reducing the risk. *Neisseria gonorrheae* colonization of the cervix is associated with

preterm delivery. Treatment to eradicate gonorrhea has been found to be beneficial in reducing the risk of PPROM.

Bacteria vaginosis in high risk patients such as those with PPROM or preterm birth in a prior pregnancy confers a 19 – 49% risk of PPROM recurrence if not eradicated with antibiotics.[21]

Puerperal sepsis may also occur after prolonged premature rupture of membranes. This is supported by the observation that many women with Bacteria vaginosis get PROM and fetal membranes get infected leading to choriamnionitis [21].

Problem Statement

Premature rupture membranes pose a great risk of having premature babies who in our circumstance may not survive. This is due to the fact that we do not have adequate facilities to care for those children who are born before 30 weeks of gestation.

At Muhimbili National Hospital PPROM occurs in about 4% of all pregnancies[22, 23]. This results in about majority of premature admission in the neonatal ward accounting for about 25 – 40% of admission in the neonatal ward. (Neonatal database)

Two studies conducted at MNH have shown the prevalence of PPROM ranges from 3 – 4.5%. But in these studies the microbial causes of premature rupture of membranes were not investigated[22, 23]

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Rationale of the Study

In Muhimbili National Hospital there is no data that shows microbial causes of premature rupture of membranes in admitted pregnant patients. This has led to empirical use of antibiotics which sometimes involve irrational prescription.

This study was done at this particular moment with the intention to address this gap. Knowing the microbial pattern of premature rupture of membranes and rational use of antibiotics is very important in the management of PPROM so as to reduce the morbidity and mortality associated with PPROM.

There is also a limited clarity on the gestation age at which PPROM occurs in our hospital. This study helped to shed light on this aspect.

In addition knowing the bacterial causes and their susceptibility to antibiotics will assist clinical management of PPROM where you don't have laboratory for doing culture and sensitivity test.

Broad Objective

To describe micro-organism associated with preterm premature rupture of membranes and their antimicrobial sensitivity patterns.

Specific Objective

1. To identify the micro –organisms associated with PPROM /PROM at MNH
2. To determine the antimicrobial susceptibility pattern of microorganism isolated.
3. To determine the gestation age at which PPROM occur commonly at MNH

METHODOLOGY

Study Design

This was a descriptive cross sectional study

Study Population

It included all pregnant mothers admitted in the labour ward due to PPROM/PROM.

Study Area

This study was conducted in the labor ward at MNH from April 2006 – October, 2006. MNH is the largest referral and teaching hospital in Tanzania. It caters for the population of about 3 million Dar es Salaam residents. The maternity unit has a capacity of 120 beds and it receives referrals from the three Municipal hospitals in Dar es Salaam namely Ilala, Temeke and Mwananyamala.

Sample Size;

This was calculated from the EPI info programme version 6 using the formula comparing 2 proportions. The following parameters were used in calculation.

Exposure in not ill group = 20%

Confidence interval = 95%

Power of the study = 80%

Ratio of exposed and not exposed 2:1

Risk ratio = 2.0

The calculation sample size = 330

Definition of terms

Premature rupture of membranes was defined as ROM when Gestation age is at 37 weeks or more before onset of labour.

PPROM is rupture of membranes before onset of labour at less than 37 weeks of gestation

Management Protocol in Muhimbili.

The Management of PPROM below 34 in MNH involves the confirmation of diagnosis by sterile speculum examination. Patients are given antibiotics Ampicillin and Flagyl. If they are below 34 weeks they are given corticosteroids. If more than 34 weeks they are induced with Oxytocin or Misoprostol.

Identification of patients with PPROM/PROM

All pregnant women admitted in the labour ward pass through admission room for evaluation. All women admitted with complaints of draining were examined to confirm the diagnosis. The diagnosis of draining was done by the principal investigator and the residents who were on call on particular day. The patients who were admitted at night were also examined and the specimen taken to the lab early the next morning.

This was confirmed by using a sterile speculum and observing for fluid coming from the cervix and pool of amniotic fluid in the vagina. If this was not possible Nitrazine paper was used for testing the vaginal pH. Gestational age was calculated from the last normal Menstrual Period and ultrasound. If not available booking fundal height was used to estimate the gestational age.

Exclusion criteria

1. Any patient who was on Antibiotics in the previous one week.
2. Those who have been diagnosed to have cervical incompetence.
3. Patients with Polyhydramnios as they are prone to get premature rupture of membranes.
4. Patient with malpresentation that included those with oblique lie, transverse lie and breech.
5. Multiple pregnancies.
6. Patient with covert chorioamnionitis

Data collection

Data was collected by the use of questionnaires after obtaining informed consent which was administered by research assistants and the investigator. Important

Obstetric data relevant for the study was obtained from the antenatal card and entered into the questionnaire. This included age, parity, source of income, marital status. The time of rupture of membranes was recorded according to the mothers' information.

This was done by the investigator with assistance from research assistants who were given training on how to include the patients. The research assistants were trained during the pre-test of the questionnaire.

Collection of specimen

In this study the following specimen were collected: endocervical swab, high vaginal swab, for culture and sensitivity and swab for Bacterial Vaginosis from each patient. A sterile Cuscos speculum was used to expose the cervix. A sterile swab stick was used to collect the endocervical secretion and high vagina, swabs, which were put immediately in a capped Amies transport media and sent to the laboratory for microbiological analysis. For Bacterial vaginosis a swab was taken and rolled on a glass slide and gram stain was done and diagnosis was made using the Nugent's criteria as seen in the table

below. Wet preparation slides were also made and examined for Candida and Trichomonas vaginalis.

Bacteria Vaginosis was evaluated by using Nugent Criteria for BV. A score of more than 7 indicate presence of bacterial vaginosis.

Table 1: Scoring Criteria for Bacterial Vaginosis

SCORING OF GRAM'S STAINS.*		
MORPHOLOGIC TYPE†	QUANTITY‡	SCORE
Large gram-positive rods	4+	0
	3+	1
	2+	2
	1+	3
	0	4
Small gram-variable or gram-	0	0
negative rods	1+	1
	2+	2
	3+	3
	4+	4
Long, curved gram-variable rods	0	0
	1+ or 2+	1
	3+ or 4+	2

* The method used was described by Nugent et al 1991.

† A gram-variable rod is a bacterial morphotype that is not consistently gram-positive or gram-negative.

‡ Five oil-immersion fields were examined. The score 4+ denotes more than 30 per oil immersion field, 3+ denotes 5 to 30 per oil-immersion field, 2+ denotes 1 t0 4 per oil-immersion field, 1+ denotes fewer than 1 per oil-immersion field and 0 denotes none seen.

Culture of Endocervical / High Vaginal swabs

Endocervical swabs were cultured on blood agar, MacConkey agar and cooked meat media. Two plates of blood agar were inoculated, one incubated aerobically and the other anaerobically at 35 – 37^{0}C. Another specimen was inoculated on MacConkey agar and inoculated aerobically at 35–37^{0}C overnight. Another specimen was inoculated in cooked meat media and incubated at 35 – 37^{0}C and sub cultured as recommended at 24, 48 and 72hrs.

Identification of Microbial Isolates

Isolates obtained after culture of the various specimens were identified as much as possible to species level using conventional microbiological techniques. However some isolates were not identified to their species level because of unavailability of the required reagents

Antimicrobial susceptibility pattern testing

Kirby – Bauer National Committee for Clinical Laboratory Standards (NCCLS) modified disc diffusion technique was used in testing antimicrobial sensitivity pattern of the isolates. Highly stable Rosco Diagnostic tablets (Neo-Sensitabs) were used as

standard antimicrobial agents in this study. Using interpretation chart, the zone of each antimicrobial agent was reported Resistant denoted by R or Sensitive denoted by S. The following antimicrobial discs were used for susceptibility testing: ampicilin, gentamicin, chloramphenicol, ceftriaxone, ceftadizime, amoxyl-clavunate and cotrimoxazole. Erythomycin discs were not available during the study period.

Data analysis

The data was entered in a computer by using EPI INFO programme version 6 of 2001, version for Epidemiology and Disease Control, Atlanta, Ga, USA. Analysis was done frequency tables and tests for significance was used. Biostatistician was consulted on data analysis

Limitation

There was unavailability of media to isolate organisms such as Chlamydia, Bacteroides due to lack of financies. Also there was lack of discs for antibiotic sensitivity test. This would also have affected the prevalence of the microbial organisms identified. History was relied in estimating gestational age rather in some of the patients than using ultrasound which is more accurate

ETHICAL CONSIDERATION

Ethical clearance to do the study was granted by MUCHS higher degree ethical committee after addressing the following ethical issues.

Participants of the study were informed about the importance of doing the study as its purpose was to improve future management of the complications and its prevention. Patients signed an informed consent before participating in the study.

In all cases appropriate treatment was given promptly after communicating to the doctor taking care of patient.

Results

From April 2006 to October 2006 a total of 4481 pregnant mothers were admitted in the labor ward of Muhimbili National Hospital. During this period a total of 341 patients had rupture of membranes before onset of labour. Out these 121 were of gestational age less than 37 and 220 were at gestation age of 37 weeks or more. Of the 121 who had PPROM 11 patients were excluded from the study for various reasons. Two had cervical cerclage, two had polyhydramnios (confirmed by ultrasound), three had used antibiotics prior to the admission and the rest (4) had vaginal examination done before they were enrolled. The analysis was based on 110 patients who had PPROM and 220 patients who had PROM. The prevalence of PPROM was 2.7% (121/4481) and PROM was about 5% (220/4481). The overall prevalence was 7.7%. Most of the women were admitted from home.

Table 2 shows the socio demographic characteristics and obstetric characteristics. More than 50% were primigravidae. The prevalence of both PPROM and PROM decreased with increasing parity. The prevalence was about 4 times in primigravidae compared with who were para 4 or more.

The median age was 23 years (16 -38). 49.1% and 53.2% in both groups respectively were in the age group of 20 -30 years. Those who attended primary school were the majority 76.4% in PPROM while in PROM group was 72.7%. Most women depended on their husbands for the source of income.

About three quarters of the women were married

Table 2: Sociodemographic, Obstetric Characteristics

	PPROM N=110(%)	**PROM** N=220(%)
Age		
<20	36(32.7)	62(28.2)
20 – 30	54(49.1)	117(53.2)
> 30	20(18.2)	41(18.6)
Marital status		
Single	26(23.6)	61(27.4)
Married	84(76.4)	159(72.6)
Gravidity		
1	65(59.3)	123(55.9)
2	19(16.9)	45(20.3)
3	11(10.2)	30(13.6)
> 4	15(13.6)	22(10.2)
Education Status		
None	19(17.2)	26(11.8)
Primary	84(76.4)	160(72.7)
Secondary	7(6.4)	34(15.5)
Source of Income		
Relatives	18(16.4)	37(16.8)
Husband	71(64.5)	120(54.5)
Employed	15(13.6)	23(10.5)
Business	6(5.5)	40(18.2)

Table 3: Gestational Age at Rupture of Membranes

Weeks	N	Percentage
< 28	5	1.5
28 – 32	16	4.8
33 – 36	89	27
> 37	220	66.7
Total	330	100

Table 3 summarizes the gestational age at which rupture of membranes occurred. The majority of patients in the PPROM group had rupture of membranes between 33 – 36 weeks (27%) but less than 2% occurred below 28 weeks of gestation. About 66.7% had ROM after 37 weeks.

Table 4: Distribution of Microorganism according to gestational age.

Gestational Age	Microbial Isolates						
	E.Coli	Klebsiella	Candida albicans	Streptococcus	N.gonorrheae	Staphylococcus	Trichomonas vaginalis
<28	2	1	1	-	-	2	4
28-32	11	7	2	2	2	-	2
33-36	2	4	2	1	-	-	1
>37	10	8	2	1	-	-	3
Total	**25**	**20**	**7**	**4**	**2**	**2**	**10**

Table 4 summarizes the distribution of microorganisms according to gestational age. Most of the microorganisms were isolated in gestational age 28 - 32. Two mothers had Neisseria gonorrhea in the gestational age of 28 – 32. *Escherichia coli* were found in 15

patients with gestational age less than 37 weeks while 10 were in the gestational age of more than 37 weeks.

Table 5: Micro-organism detected during the study

	PPROM		PROM	
	N	(%)	N	(%)
E.Coli	15	(30)	10	(38.5)
Klebsiella	12	(24)	8	(30.2)
Candida albicans	5	(10)	2	(7.7)
N. gonorrhea	2	(4)	-	-
B-Streptococci	4	(8)	2	(7.7)
Staphylococcus aureus	2	(4)	-	-
Trichomonas vaginalis	7	(14)	3	(11.5)
Streptococcus pyogenes	3	(6)	1	(3.6)
Total	50	(100)	26	(100)

Table 5 shows distribution of the micro-organisms isolation in PPROM and PROM From Fifty PPROM group, pathogens were isolated from the PPROM group giving a recovery rate of 45.5%. From PROM group 26(11.5%), pathogens were isolated.. The commonly isolated organism in both groups was *Escherichia coli* and *Klebsiella spp* were found in 54% patients in PROM group but accounted for 68.7% in the PROM group .In this study *Neisseria gonorrhea* (4%) and *Staphylococcus aerus* (4%) were isolated in the PPROM group only.

Table 6: Bacterial vaginosis isolated during the study

	PPROM		PROM	
	N	**(%)**	**N**	**(%)**
BV +ve	41	(37.2)	52	(23.6)
BV -ve	69	(62.8)	168	(76.4)
TOTAL	**110**		**220**	

Table 6 shows the distribution of Bacterial vaginosis

In the PPROM group 41(37.2%) had *Bacterial vaginosis* while in the PROM group 52(23.6%) had *Bacterial vaginosis.* The difference observed was statistically significant (p=0001).

Table 7: Antimicrobial susceptibility patterns of isolates from PPROM and PROM patients

		Ampicillin		Gentamicin		Cotrimoxazole		Amoxclav		Chloramphenical		Cefuroxime		Ciprofloxacin		Ceftriaxone	
Organisms		N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
E.Coli	S	7	28	21	84	0	0	19	76	8	42.9	21	84	22	88	25	100
	R	18	72	4	16	25	100	6	24	17	57.1	4	16	3	12	0	0
Staphylococcus	S	0	0	2	100	1	50	2	100	1	50	1	50	2	100	2	100
	R	2	100	0	0	1	50	0	0	1	50	1	50	0	0	0	0
S. pyogenes	S	1	25	4	100	4	100	4	100	1	25	3	75	4	100	4	100
	R	3	75	0	0	0	0	0	0	3	75	1	25	0	0	0	0
Neisseria	S	0	0	0	0	0	0	2	100	0	0	2	100	2	100	2	100
	R	2	100	2	100	2	100	0	0	2	100	0	0	0	0	0	0
Klebsiella	S	6	30	12	60	2	10	14	70	0	0	13	65	20	100	14	70
	R	14	70	8	40	12	90	6	30	20	100	7	35	0	0	6	30

Table 7 shows antimicrobial susceptibility pattern of the isolates from both groups. Escherichia coli was sensitive to Gentamicin (84%), Cefuroxime (85.8%) Ceftriaxone (100%) but it was resistant to Ampicilin (28%) and Chloramphenical (42.9%). Klebsiella showed resistance to Ampicillin and Cotrimoxazole (90%) and it was 100% resistant to Chloramphenical. Sensitivity was 100% to Ciprofloxacin. And 60% of the isolatestoGentamicinStaphylococcus aureus was resistant to Ampicilin (100%) but was sensitive to Cefuroxime, Ceftriaxone and Ciprofloxacin. While Streptococcus pyogenes was resistant to Ampicilin and Chloramphenical, it was sensitive in Gentamicin, Ceftriaxone and Cefuroxime. Neisseria gonorrhea was resistant to Ampicilin, Gentamicin and Chloramphenical; it was sensitive to Ceftriaxone, Ciprofloxacin by 100%.

Discussion

During the study period the prevalence of PPROM and PROM at Muhimbili National Hospital was 2.7 % and 5% respectively. This is similar to what was seen in earlier studies by Bwire and Hamudu [22, 23]. Worldwide the prevalence is between 3 – 5%.[1, 2]

In this study the microorganisms isolated from the cervical secretions were investigated comparing those who had PROM and PPROM. The bacterial flora of the female reproductive system is the central point of the study of several pathologies in Gynaecology and Obstetrics. In PROM, the determination of the cervical microbiology is of the utmost importance owing to the ascending aetiology of infection. Ascending infection through the cervix causing Neonatal morbidity and mortality is the second major problem after prematurity when pre-labour rupture of membranes occur[24]. Maternal mortality and morbidity occur due to endometritis and sometimes sepsis from the ascending infection.

The socio-economic status e.g. marital status, social dependence did not show any association with premature rupture of membranes. These results are in agreement with the study done in Brazil.[24] During the study period all mothers were self referral from home.

In this study it has been revealed that E.coli, Klebsiella spp, Staphyloccocus aureus, Candida, Trichomonas vaginalis were isolated from both endocervical and high vaginal

swabs. Other organism included B-streptococci, Neisseria gonorrheae. The most frequently isolated organism was E.coli (30%) followed by Klebsiella (24%). This is similar to a study done in Brazil and in Nigeria[20, 24].

But in the study done in Nigeria E coli was not isolated. Also in that study B streptococcus was not isolated. In our study only 4% of the B-streptococcus was isolated. This is different from other studies where by the colonization of B-streptococcus was found to be 5-30%. This difference can be explained by the fact that we took only high vaginal and endocervical swabs whereas screening for the organism is a combination of low vaginal and rectal swabs. With rectal swabs the detection rate increases[11, 25].

Candida albicans was also isolated in this study. This is also in agreement with other studies reported previously[24, 25]. However Candida albicans has not been specifically implicated in premature rupture of membranes. Other organisms that were also isolated in this study were Streptococcus pyogenes and Trichomonas vaginalis. T. vaginalis has been linked with PROM in previous studies[24, 25].

Staphylococcus aureus was isolated in both groups. This is also true in other studies[18, 20, 24, 26]. This finding has shown that S. aureus is possibly implicated in the pathogenesis of PPROM. According to literature Staphylococcus produce phospholipase A and C which esterify the arachidonic acid of membranes which in turn is metabolized for

cyclooxygenase pathway forming prostaglandins. These reactions cause an increase in the uterine cavity, leading to membrane weakness[24].

In this study Bacterial vaginosis was also isolated. This is also in line with other studies where by screening reveals a 30% isolation of bacterial vaginosis in patients with PPROM[13]. In our study it was 36% among the PPROM and 23% among the PROM group. This can be explained by the fact that Bacterial vaginosis has not been associated with PPROM. It has mainly been associated with preterm birth rather than PPROM

Other organisms could not be isolated due to the non availability of medium for their isolation. Similar reasons can be adduced for the non-isolation of Chlamydia organisms. The lack of these facilities may have an influence on the reduced prevalence of microbial organisms isolated in this study and therefore overestimate others.

Identification of anaerobic bacteria such as Bacteroides was not done in this study due to absence of specific medium. *Bacteroides fragilis* has been linked to PPROM[24]. Thus consideration for Metronidazole where therapy is needed in cases of PPROM should remain in practice.

Recent evidence has shown conclusively that the use of antibiotics in PPROM is associated with significant reduction in chorioamnionitis and delay in delivery[3, 25].

Recent evidence has shown conclusively that the use of antibiotics in PPROM is associated with significant reduction in chorioamnionitis and delay in delivery[3, 25].

Antibacterial therapy has been used as prophylaxis for prevention of infection following premature rupture of membranes. According to the Cochrane review (2003) Erythromycin is the drug of choice. Unfortunately in this study Erythromycin discs were not available for checking sensitivity pattern. In our set up we use Ampicilin and Metronidazole during treatment of PPROM. In our institution it has not been shown why we use the above medication for treatment of patients with PPROM.

In this study antibiotics such as gentamicin and Ceftriaxone were shown to be very effective against most of the organisms. *Klebsiella* and *E.coli* was seen to be resistant to Ampicilin. Also *Streptococcus pyogenes* and Group B-*Streptococci* were resistant to Ampicilin. This was also seen in other studies[26]. This was also found in the study done here by Makuwani in 2003 who investigated the microbial pattern of puerperal sepsis. Majority of the organisms were susceptible to ciprofloxacin, ceftriaxone, and ceftadizime in the range of 60 – 80%. But drugs such as Ciprofloxacin are not recommended for use during pregnancy[20].

Klebsiella and *E.coli* were resistant to Chloramphenicol. This was also seen in the study done by Makuwani and Ndugulile who studied Patterns and microbial susceptibility of bacteria isolates causing pyogenic infection[30]. *E.Coli, Streptococci, Klebsiella* and *N. gonorrheae* were also sensitive to Amoxy-clav. But this drug is not recommended because it has been shown to cause neonatal necrotizing enterocolitis[27].

Conclusion

1. This study has shown that bacteria pathogens are associated with PPROM and PROM. In our set up *E. coli* and *Klebsiella* were frequently isolated. Bacterial vaginosis was also isolated. It was established that *Staphylococcus aureus* and *Neisseria gonorrhea* were not isolated in patients with PROM but there were isolated in the patients with PPROM

2. In terms of microbial sensitivity majority of organisms were sensitive to ceftriaxone, gentamicin and ciprofloxacin in this study.

Recommendations

1. The protocol of use of antibiotics in our set up should be put forward. Emphasizing that Ampillicin is resistant to most of microorganisms. Ceftriaxone is recommended to be used in our set up. The price of Ceftriaxone is reduced now. It is now readily available at Muhimbili National Hospital.

2. Routine endocervical swabs should be taken in those patients with PPROM remote from term.

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Appendix 1.

Questionnaire

Interview

1. Serial Number: _____
2. Date of Admission: _____
3. Name of patient: _____
4. Age of patient: _____
5. Referred from: _____
6. Marital status
   a. Single
   b. Divorced
   c. Married
   d. Cohabiting
7. What is your parity
   a. 1
   b. 2
   c. 3
   d. > 4
8. Level of education
   a. Not attended formal education
   b. Primary school

c. Secondary School
d. College education

9. Occupation
   a. Housewife
   b. Peasant
   c. Business
   d. Employed
   e. Others: _____

10. Attendance to clinic
    How many times

11. Date of rupture of membranes _____ time _____

12. Gestational age at PROM /PPROM: _____

13. Duration of rupture at the time of Admission: _____

14. Past obstetric complications
    History of PROM Yes: _____ No: _____
    History of Preterm delivery Yes: _____ No: _____
    History of prelabour ROM (at term) Yes: _____ No: _____

15. Antenatal complications in the index pregnancy
    a. P.V. bleeding Yes: _____ No: _____
    b. Abnormal vaginal discharge Yes: _____ No. _____

16. Were you given any antibiotics in the last 7 days?
    a. Yes
    b. No
17. HIV screening test?

    Was it done antenatally?

    a. Yes
    b. No.

If yes what is the result

    a. Positive
    b. Negative

Section B: Investigations

18. Specimen collected: [Tick V for Yes and X for No]
    a. High Vaginal Swab: _____
    b. Endocervical Swab_____
    c. Swab for bacterial vaginosis _____

Appendix II.

Patient's Information Form

We are doing a research on premature rupture of membranes among pregnant mothers who come to deliver at our labour ward.

The aim is to know the commonest micro-organisms and their sensitivity patterns associated with Premature rupture of membranes. This will help us to treat the problem when it occurs so mothers can have safe and good outcome of their pregnancies.

Specimen will be taken from your private parts and will be sent to the laboratory for analysis. This will involve the use of A sterile Cuscos speculum which will be introduced to your vagina so as to collect the specimen. This could be a little bit uncomfortable but care will be taken not to feel bad.

You may decide not to participate in this study. This will not affect the quality of care you are supposed to get from us as you will be attended just as any other patient.

The exercise is confidential. The outcome of the research will help us to know how better to deal with this condition.

The service is free of charge. If you have any doubts you can ask Dr Furaha August who is the main researcher or the nurse in charge of the labour ward.

Thank you for your cooperation.

Appendix III.

Consent Form

I…….. I voluntarily agree to participate in this study of Premature Rupture of Membranes. I also testify that I truly understand the purpose of this study, as its importance is to improve reproductive health care

The Undersigned……..

Witnessed by……..

Signature……

Appendix IV

Fomu ya taarifa kwa mgonjwa

Tunafanya utafiti kuhusu kutoka maji kabla ya kupata uchungu.

Nia ya utafiti huu ni kujua vijidudu vinavyo sababisha hali hii na kujua pia ni dawa gani inayotibu vijidudu hivi. Hii itasaidia kutibu ugonjwa huu pindi linapotokea ili kulinda usalama wa mama na mtoto kabla ya kujifungua

Kipimo kitatolewa kutoka sehemu za siri na kupelekwa maabara kwa uchunguzi. Hii itahusisha kuweka kifaa fulani sehemu za siri na kuchukua kipimo hicho. Inawezekana ukapata maumivu kidogo.

Unaweza kukubali au kukataa kujiunga na utafiti huu. Hii haitaondoa haki yako ya kupata matibabu unayotakiwa kupata. Utahudumiwa vizuri kama wagonjwa wengine

Zoezi hili ni la siri. Utafiti utatusaidia kujua njia bora zaidi ya kutibu tatizo hili

Huduma hii haina gharama yoyote kwako. Kama una mashaka yoyote muulize Dr Furaha August ambaye ndio mtafiti mkuu au sista ambaye ni mkuu wa wodi hii.,

Asante sana kwa ushirikiano wako

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