

Bacteria Isolates Sensitivity Pattern and Pregnancy Outcome of Preterm Pre-Labour Rupture of Fetal Membranes in Ile-Ife, Nigeria

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Abstract

Background: Preterm prelabour rupture of fetal membranes (PPROM) is a significant cause of perinatal morbidity and mortality. It is the leading cause of preterm deliveries and recent studies have shown that lower genital tract infections are major aetiological factors. Antimicrobial therapy is paramount in the management of PPRM, however, emergence of multidrug-resistant (MDR) pathogens has significantly challenged effective management of these patients. The primary aim of the study is to identify antibiotics resistance pattern of bacterial isolates in cases of PPRM in OAUTHC, Ile-Ife. **Methods:** This was a hospital-based longitudinal descriptive study which involved 120 pregnant women with preterm prelabour rupture of fetal membranes. Demographic and clinical data were obtained from the patient's case notes. Samples were taken from the egressed amniotic fluid for microbiological analysis. Data were analyzed using statistical package for social sciences (IBM-SPSS) version 20.0. A p-value of 0.05 was taken as statistically significant. **Results:** The bacterial isolates were predominantly *Staphylococcus aureus* (29.8%), *Escherichia coli* (25.2%) and *Klebsiella species* (16.1%). Of the 131 bacteria, 73 (55.7%) were MDR showing high resistance to many first-and second-line antibiotics, they however, showed high susceptibility to piperacillin-tazobactam, amikacin and meropenem. Out of the 120 women that were recruited, 23 (19.2%) developed puerperal sepsis which was the cause of mortality recorded (n = 2; 1.7%). Thirty-four (28.6%) of the total 119 babies delivered alive, developed clinical sepsis and 23 (19.3%) were culture positive cases. MDR blood stream infection

was responsible for four (40%) of the total neonatal mortality. **Conclusion:** Most of the organisms cultured in this study were multiple drug resistant *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella species* of bacteria with great potential to cause maternal and neonatal infections. They are sensitive to piperacillin-tazobactam, amikacin, meropenem, and Cefuroime. This is evidence to support the need for review of antibiotic treatment protocols for management of PPRM.

Keywords

Preterm Prelabour Rupture of Membranes, Bacterial Isolates, Antibiotic Resistant Pattern

1. Introduction

Preterm prelabour rupture of fetal membranes (PPROM) is defined as rupture of membranes after the age of viability, but before 37 weeks gestation.¹ Approximately 3% - 4.5% of all deliveries are complicated with PPRM [1]. Important events that herald the onset of labour at term are programmed cell death and activation of catabolic enzymes such as collagenase and mechanical forces resulting in rupture of fetal membranes [2]. PPRM occurs probably due to the same mechanisms by premature activation of these pathways. However, PPRM also appears to be linked to underlying pathologic processes, most likely due to inflammation or infection of the membranes [3].

PPROM complicates on the average; about 3% - 4.5% of all pregnancies [1], and its risk factors include low socio-economic status, low body mass index, trauma, tobacco use, polyhydramios, multiple gestation, urinary tract infection, cervical cerclage, vaginal bleeding at any time in pregnancy, amniocentesis and previous history of PPRM [4] [5]. It is a significant cause of preterm delivery which is the leading cause of perinatal morbidity and mortality [2]. PPRM accounts for a perinatal mortality rate of as high as 60% - 80%, and 30% - 40% of preterm deliveries [6] [7]. Preterm delivery is a foremost cause of perinatal mortality, and it is associated with enormous neonatal complications including hypothermia, necrotizing enterocolitis, intraventricular haemorrhage, respiratory distress syndrome (RDS) and sepsis [8]. PPRM is also a major risk factor for long-term sequelae such as chronic lung disease, neurosensory impairment, developmental delay and cerebral palsy. There are contemporary data and evidences linking perinatal infections to neurological complications [9]. PPRM also confers a huge responsibility on the obstetricians, including the need to confirm the diagnosis and establish the best option of management. This may however not be easily achieved because of the crucial need to maintain the critical balance between the complications of prematurity and the associated risks of chorioamnionitis with sustained continuity of pregnancy.

Genital tract infection can be associated with PPROM as either cause or consequence; although aetiology of PPROM is multifactorial [9] [10], the role of microorganisms colonizing the lower genital tract in precipitating PPROM and preterm labour has been widely discussed. Available reports implicate urogenital tract infections as a major contributor to the aetiology of PPROM [4] [11]. Increasing evidence has linked some clinical risk factors, amniotic fluid microbiology and membrane histology to PPROM. Infection preceding PPROM is often subclinical and thought to be ascending from lower genital tract. Following rupture of the membranes, ascending bacteria invasion often leads to intrauterine infection in absence of antibacterial therapy. Several studies have shown that women with low social economic status are at higher risk of PPROM and more likely to develop chorioamnionitis as a complication of PPROM [12] [13]. A wide spectrum of microorganisms is linked to PPROM and they are mostly vaginal flora. In addition, microorganisms, such as *Mycoplasma hominis* and *Ureaplasma urealyticum* have been isolated more from the amniotic fluid of women who experienced preterm delivery caused by PPROM [14].

The primary aim of the study is to determine the bacterial isolates implicated in preterm prelabour rupture of fetal membranes and their sensitivity patterns. The secondary objective is to determine the maternal and neonatal outcomes of PPROM among the study population with the aim of generating evidence-based data for management of pregnant women with PPROM in our environment and other resource-constraint settings.

2. Methods

2.1. Study Design and Setting

This hospital-based longitudinal study was conducted at the Department of Obstetrics, Gynaecology and Perinatology and the Department of Medical Microbiology and Parasitology of the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State, Nigeria between March 2023 and February 2024. The hospital comprises two arms offering tertiary healthcare: the Ife Hospital Unit in Ile-Ife and the Wesley Guild Hospital in Ilesa. The Teaching Hospital Complex serves as a referral centre for primary and secondary level healthcare centres within Osun, Ondo, and Ekiti State. The two obstetrics units conduct an average of 2850 deliveries every year with incidence of PPROM of 2.4% [15], and antenatal booking clinic attendance of an average of 3650 per year. All pregnant women admitted for PPROM between 28 weeks and 36 + 6 weeks gestational age at the Ife Hospitals Unit and the Wesley Guild Hospital, Ilesa.

2.2. Sample Size and Sampling Technique

The minimum sample size was calculated using the formula for determining sample size for single proportion with $P = 0.24$ (an incidence of 2.4% for PPROM in a previous study in Ile-Ife, Nigeria and 10% attrition rate). A total of 120 patients were recruited for this study from the two tertiary health facility units of the hos-

pitals by purposive sampling method. All consecutive eligible PPRM admissions were enrolled until the sample size was achieved,

2.3. Data Collection and Tools

Consented pregnant women with PPRM between 28 weeks and 36 + 6 weeks of gestation were included in this study. The patients excluded were non-consenting pregnant women, those that have had vaginal examination prior to presentation, patients with suspected chorioamnionitis at admission, patients with PPRM who had antibiotics in the previous one week prior to the onset of symptoms, patients with polyhydramnios, pregnant women with associated antepartum haemorrhage. Patients with higher order pregnancies and those with ultrasound diagnosed congenital anomaly. Patients were recruited on admission at the antenatal wards after the diagnosis of PPRM had been established. Patients were thoroughly informed about the study and written informed consent was obtained from each of them. After recruitment, demographic and clinical data were collected by the use of a purpose– designed proforma. Relevant information obtained from the patient’s case notes include the age, parity, vital signs, marital status and history of PPRM in her previous pregnancy. The social economic class of the patient was determined according to Olusanya *et al.* [16] and documented in the proforma. The time of rupture of membrane was recorded according to the patient’s information.

2.4. Collection of Specimens

On admission with the aid a sterile Cusco’s speculum, samples of egressed amniotic fluid were taken with swab sticks and sent for microscopy, culture, and sensitivity.

A disposable sterile bivalve Cusco’s speculum was used to expose the cervix, swab stick was used to collect amniotic fluid into peptone water and sent to the microbiology laboratory for processing within 3 hours of sample collection. Endocervical swabs were taken from the same group of mothers who developed features of puerperal sepsis within 14 days post-delivery for microscopy, culture, and sensitivity.

2.5. Identification of Patients with PPRM

Gestational age was calculated from the first day of her last menstrual period and early ultrasound scan. All pregnant women between the gestational ages of 28 weeks and 36 + 6 week with complaint of drainage of liquor had a sterile vagina speculum examination done to confirm the diagnosis. The diagnosis was confirmed by observation of egress of fluid from the cervix and pool of amniotic fluid at the posterior vaginal fornix; if not obvious a Valsalva manoeuvre was done. The fluid was confirmed with Amnisure® (Placental α 1-microglobulin) which makes the results available within 10 minutes with sensitivity approaching 99%. On admission with the aid a sterile Cuscos speculum, samples of egressed amniotic fluid

were taken with swab sticks and sent for microscopy, culture and sensitivity.

A disposable sterile bivalve Cusco speculum was used to expose the cervix, swab sticks were used to collect amniotic fluid which was put in a capped Amies Transport Media and sent to the microbiology laboratory for processing within 4 hours of sample collection.

2.6. Clinical Management

The patients recruited into the study were commenced on antibiotics—Erythromycin 500 mg 6 hourly for ten days; if the gestational age is below 34 weeks, they will be given corticosteroid—intramuscular dexamethasone 12 mg 12 hourly for 24 hours to aid fetal lung maturity.

Tocolytics was not employed except in order to gain time for antepartum corticosteroid to exert its maximum benefit. Oral Nifedipine was used for tocolysis in such instance of patients with gestational age less than 34 weeks.

2.7. Bacteria Isolation

The amniotic fluid specimen and endocervical swabs respectively collected from mothers with PPRM and puerperal sepsis were inoculated on 5% Sheep Blood Agar, and MacConkey agar (Oxoid, England) and it was incubated aerobically at 35°C - 37°C for 18 - 24 hours. Bacterial isolates from these mothers and their neonates who developed h were stored in the -80°C ultra cold freezer.

2.8. Identification of Bacteria Isolates

Isolates obtained from the culture of specimens from mothers with PPRM and puerperal sepsis as well as those achieved in the laboratory from blood culture of their neonates were identified by Gram stain, standard biochemical tests (including catalase test, coagulase test, oxidase test, citrate test, urease test and other sugar fermentation tests) and use of Microbact GNB24E (Oxoid, England) which is a standardized micro-substrate system for identification of Enterobacteriaceae and common miscellaneous Gram—Negative bacilli. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 were used for quality control of the biochemical tests.

2.9. Antimicrobial Susceptibility Testing

The antibiotic susceptibility testing was done by modified Kirby-Bauer disk diffusion technique as recommended by Clinical and Laboratory Standard Institute (CLSI) [17].

Pure isolates were transferred from blood and MacConkey agar to nutrient agar from where inocula were prepared for antibiotic susceptibility testing. Discrete colonies of isolates were picked from less than 24 hours old cultures on nutrient agar and emulsified into tubes containing 5 mls of sterile saline solution to match 0.5 McFarland turbidity standards. Sterile cotton swabs were dipped into the inocula and then pressed firmly against the side of the tubes above the level of the

liquid to remove excess fluid. The wet swabs were used to make lawns (confluence inocula) on Mueller-Hinton agar (MHA) by swabbing in three directions. The swabbed plates were allowed to dry at room temperature and a set of six antibiotics discs were placed evenly on each of the swabbed MHA Petri dish plate using sterile forceps.

After 18 hours of incubation, the diameter of the zone of inhibition around each antibiotic disc was measured and recorded in mm. The zones of inhibition of antibiotics were interpreted as “sensitive”, “intermediate” and “resistant” in accordance with CLSI guidelines [17]. Isolates with intermediate sensitivity were regarded as “resistant”.

2.10. Data Analysis

Data analysis was performed using IBM-SPSS version 20. Quantitative variables were expressed as mean + standard deviation SD while qualitative variables were expressed as percentages. Comparison of mean values was done using student t-test, for continuous variables and chi-square for categorical variable. A P-value of ≤ 0.05 was considered to be statistically significant.

2.11. Ethical Consideration

Ethical approval, with protocol number ERC/2022/09/17, was obtained from the Ethics and Research Committee of The Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife (Protocol number - ECR/2022/09/17).

All participants were fully informed about the study and they were informed that they reserve the right to withdraw for whatever reasons at any stage of the study without any penalty. Confidentiality was maintained by, identifying each patient with their initials and study identification numbers. The details of the data from this study were stored on personal computer with the password known only to the principal investigator

3. Results

3.1. Socio-Demographic and Clinical Characteristics of Patients

During the study period, a total of 2920 pregnant women presented for deliveries out of which 129 had PPRM giving an overall incidence of 4.4%. Most of the 120 patients recruited for the study fall within the expected age range 21 - 30 (72; 60%) and 31 - 40 years (35; 29.2%), and primigravidae (41; 34.2%) accounted for the patients with the highest frequency, with only 1 (0.8%) patient with parity > 4. The mean parity was 2. More than a third (34.2%) of the patients were artisans. Thirty-six (30%) had no formal education, and this was closely followed by those who had primary (30; 25%) and secondary (30; 25%) education. It shows that 55.8% were Christians while Islam and others accounted for 40.8 and 3.4 respectively as shown in **Table 1**.

Fifty-one cases (42.5%) of PPRM occurred at EGA 32 - 34 weeks. For others, PPRM occurred at EGA of 28 - 30 weeks (41; 34.2%) and 35 - 36 + 6 (23; 23.3%),

mean EGA was 32 weeks. The patients were predominantly unbooked (76; 63.3%). The latency period > 7 days was 40.0%, followed by 4 - 7 days (34.2%) and 1 - 3 days (26%). About four of every five deliveries were preterm, 15.0% (n = 18) term delivery and 3.3% (n = 4) post-term deliveries as shown in **Table 2**.

The modes of deliveries were: SVD (101; 84.2%), EMLSCS (13; 10.8%) and ELCS (6; 5.0%). A total of 124 babies were delivered (116 singleton and 4 sets of twins); 78 (62.9%) were male and 46 (36.7%) were female with their birth weights less than 2.5 kg in the majority of the cases (84; 66.7%) as shown in **Table 2**.

Table 1. Socio-demographic characteristics of women with PPRM.

Socio-demographic Variables	Classification	Frequency	Percentage
Age (Years)	<20	4	3.3
	21 - 30	72	60
	31 - 40	35	29.2
	>40	9	7.5
Parity (Mean parity = 2)	0	23	19.2
	1	41	34
	2	22	18.3
	3	26	21.7
	4	7	5.8
	>4	1	0.8
Social Class	Low (4 - 5)	53	44.2
	Middle	46	38.3
	High	21	17.5
Ethnicity	Yoruba	84	70
	Hausa	11	9.2
	Igbos	21	17.5
	Others	4	3.3
Religion	Christian	67	55.8
	Islam	49	40.8
	Others	4	3.4
Marital Status	Single	12	10
	Married	106	88.3
	Divorced	2	1.7
Educational Status	No formal education	36	30
	Primary	30	25
	Secondary	30	25
	Tertiary	24	20

Continued

Occupation	Self-employed	24	20
	Artisan	41	34.2
	Government employed	24	20
	Private employed	21	17.5
	Unemployed	10	8.3
Husband Education	No formal education	24	20
	Primary	41	34.2
	Secondary	35	29.2
	Tertiary	20	16.7
Husband Occupation	Self-employed	28	23.3
	Artisan	37	30.8
	Government employed	29	24.2
	Private employed	22	18.3
	Unemployed	4	3.3

Table 2. Clinical parameters of women with PPROM.

Variables	Classification	Frequency	Percentage
Booking Status	Booked	44	36.7
	Un-booked	76	63.3
Gestational age at Admission (weeks) (Mean EGA) = 32 ± 6	28 - 32	41	34.2
	32 - 34	51	42.5
	35 - 36	28	23.3
EGA at delivery	Preterm	98	81.7
	Term	18	15
	Post term	4	3.3
Mode of Delivery	SVD	101	84.2
	EMLSCS	13	10.8
	ELSCS	6	5
Latency period/PPROM delivery interval	1 - 3 days	31	25.8
	4 - 7 days	41	34.2
	>7 days	48	40
Sex of the baby	Male	78	62.9
	Female	46	37.1

3.2. Distribution of Isolates among PPROM Cases

Seventy nine percent (n = 95) of specimens yielded at least one organism on culture; there were 67 (56%) monomicrobial culture and 28 (23%) polymicrobial cultures.

A total of 131 bacteria were isolated from 120 specimens with an average of 1.1 isolates per sample. Gram negative bacilli (GNB) were the predominant isolates

(n = 78; 59.5%), the rest were Gram positive cocci (n = 53; 40.5%). Overall, *Staphylococcus aureus* was the most common isolate (39; 29.8%), others were *Escherichia coli* (33; 25.2%), *Klebsiella* species (21; 16.1%), *Proteus* species (9; 6.8%), *Pseudomonas aeruginosa* (8; 6.1%) and *Streptococcus* species (7; 5.3%) (**Table 3**).

Table 3. Bacterial profile of PPROM.

Isolates (n; % of total)	Names of isolates	Proportion within category (%)	Total (N = 131) n (%)
GPC (n = 53; 40.5%)	<i>Staphylococcus aureus</i>	73.6	39 (29.8)
	<i>Streptococcus</i> species	13.2	7 (5.3)
	CoNS	7.5	4 (3.1)
	<i>Enterococcus</i> species	5.7	3 (2.3)
GNB (n = 78; 59.5%)	<i>Escherichia coli</i>	42.3	33 (25.2)
	<i>Klebsiella pneumoniae</i>	21.8	17 (13.0)
	<i>Klebsiella oxytoca</i>	5.1	4 (3.1)
	<i>Proteus mirabilis</i>	9	7 (5.3)
	<i>Proteus vulgaris</i>	2.6	2 (1.5)
	<i>Pseudomonas aeruginosa</i>	10.3	8 (6.1)
	<i>Enterobacter aerogenes</i>	5.1	4 (3.1)
	<i>Morganella morganii</i>	3.8	3 (2.3)

spp—species; *S. aureus*—*Staphylococcus aureus*; CoNS—Coagulase negative Staphylococci; GPC—Gram positive cocci; GNB—Gram negative bacilli.

3.3. Antibiotic Resistance Pattern of Bacteria from Cases of PPROM

Gram positive cocci showed high resistance of 73.6%, 71.7%, 56.6% and 43.3% to co-trimoxazole, ampicillin, cloxacillin and chloramphenicol respectively. On the other hand, resistance of 26.4% was shown to ofloxacin, and 18.9% to each of piperacillin/tazobactam and amikacin. Resistance of GPC to other antibiotics were: cefuroxime (34%), erythromycin (34%), gentamicin (35.8%), ceftriaxone (37.7%) and amoxiclav (37.7%) (**Table 4**).

Gram negative bacteria showed an overall high resistance to the tested third generation cephalosporins which include ceftazidime (30.8%), cefotaxime (34.3%) and ceftriaxone (37.1%). Similar resistance pattern was also shown to ciprofloxacin (30.8%) and gentamicin (37.2%). Higher resistance was to ampicillin (80%), cotrimoxazole (77.1%), amoxicillin (54.1%), and chloramphenicol (45.7%). Low resistance was however shown to meropenem (12.8%), piperacillin/tazobactam (20.5%) and amikacin (21.8%). Details of antibiotic resistance pattern of Gram-negative bacteria are shown in **Table 5**.

Seventy-three (55.7%) of the bacterial isolates were multidrug resistant (MDR) showing resistance to one or more agents in at least three antibiotics classes. They were accounted for by 33 (62.3%) of GPC and 40 (51.3%) of GNB. These MDR

bacteria were *Staphylococcus aureus* (24; 61.5%), *Klebsiella* species (11; 52.4%), *Escherichia coli* (16; 48.5%) and others as shown in **Table 6**.

Table 4. Antibiotic resistance pattern of gram-positive bacteria.

Antibiotics	<i>Streptococcus</i> spp (N = 7) n (%)	<i>S. aureus</i> (N = 39) n (%)	CoNS (N = 4) n (%)	<i>Enterococcus</i> spp (N = 3) n (%)	Total (N = 53) n (%)
Ciprofloxacin	3 (42.9)	13 (33.3)	1 (25.0)	1 (33.3)	18 (34.0)
Ceftriaxone	3 (42.9)	15 (38.5)	1 (25.0)	1 (33.3)	20 (37.7)
Gentamicin	3 (42.9)	14 (35.9)	1 (35.0)	1 (33.3)	19 (35.8)
Erythromycin	2 (28.6)	13 (33.3)	1 (25.0)	2 (66.7)	18 (34.0)
Ofloxacin	2 (28.6)	11 (28.2)	0 (0.0)	1 (33.3)	14 (26.4)
Cloxacillin	4 (57.1)	22 (56.4)	2 (50.0)	2 (66.7)	30 (56.6)
Cefoxitin	4 (57.1)	14 (35.9)	1 (33.3)	2 (33.3)	21 (39.6)
Ampicillin	7 (100.0)	28 (71.8)	2 (50.0)	1 (33.3)	38 (71.7)
Cefuroxime	3 (42.9)	13 (33.3)	1 (25.0)	1 (33.3)	18 (34.0)
Piperacillin/taz	2 (28.6)	8 (20.5)	0 (0.0)	0 (0.0)	10 (18.9)
Amoxiclav	3 (42.9)	15 (38.5)	1 (25.0)	1 (33.3)	20 (37.7)
Chloramphenicol	4 (57.1)	16 (41.0)	1 (25.0)	2 (66.7)	23 (43.4)
Amikacin	2 (28.6)	7 (17.9)	0 (0.0)	1 (33.3)	10 (18.9)
Co-trimoxazole	5 (71.4)	29 (74.4)	3 (75.0)	2 (66.7)	39 (73.6)

Table 5. Antibiotic resistance pattern of gram negative bacteria.

Antibiotics	<i>E. coli</i> (N = 33) n (%)	<i>Klebsiella</i> spp (N = 21) n (%)	<i>Proteus</i> spp (N = 9) n (%)	<i>P. aeruginosa</i> (N = 8) n (%)	<i>Enterobacter</i> spp (N = 4) n (%)	<i>M. morganii</i> (N = 3) n (%)	Total (N = 78 or 70) n (%)
Ciprofloxacin	10 (30.3)	6 (28.6)	2 (22.2)	3 (37.5)	2 (50.0)	1 (33.3)	24 (30.8)
Ceftriaxone	12 (36.4)	7 (33.3)	4 (44.4)	NT	2 (50.0)	1 (33.3)	26 (37.1)
Gentamicin	12 (36.4)	8 (38.1)	3 (33.3)	4 (50.0)	1 (25.0)	1 (33.3)	29 (37.2)
Ofloxacin	9 (27.3)	6 (28.6)	2 (22.2)	3 (37.5)	1 (25.0)	1 (33.3)	22 (28.2)
Ceftazidime	10 (30.3)	7 (33.3)	2 (22.2)	2 (33.3)	2 (50.0)	1 (33.3)	24 (30.8)
Cefotaxime	11 (33.3)	8 (38.1)	3 (33.3)	NT	1 (25.0)	1 (33.3)	24 (34.3)
Cefepime	10 (30.3)	6 (28.6)	2 (22.2)	2 (25.0)	1 (25.0)	1 (33.3)	22 (28.2)
Piperacillin/Taz	6 (18.2)	4 (19.0)	2 (22.2)	2 (25.0)	1 (25.0)	1 (33.3)	16 (20.5)
Meropenem	4 (12.1)	3 (14.2)	1 (11.1)	2 (66.7)	0 (0.0)	0 (0.0)	10 (12.8)
Ampicillin	25 (75.8)	17 (81.0)	7 (77.8)	NT	4 (100.0)	3 (100.0)	56 (80.0)
Cefoxitin	10 (30.3)	8 (38.1)	4 (44.4)	4 (50.0)	2 (50.0)	1 (33.3)	29 (37.2)
Amoxicillin	20 (60.6)	9 (42.9)	5 (55.6)	NT	2 (50.0)	2 (66.7)	38 (54.3)
Amoxiclav	13 (39.4)	6 (28.6)	3 (33.3)	NT	2 (50.0)	1 (33.3)	25 (35.7)
Chloramphenicol	14 (42.4)	10 (47.6)	4 (44.4)	NT	3 (75.0)	1 (33.3)	32 (45.7)
Amikacin	7 (21.2)	5 (23.8)	2 (22.2)	2 (25.0)	0 (0.0)	1 (33.3)	17 (21.8)
Co-trimoxazole	26 (78.8)	15 (71.4)	7 (77.8)	NT	3 (75.0)	3 (100.0)	54 (77.1)

NT—Not tested.

Table 6. Multidrug-resistant Bacteria Isolates.

Bacterial Isolates (N)		Frequency of MDR	Percentage of MDR
		33	62.3
PC (N = 53)	<i>S. aureus</i> (39)	24	61.5
	<i>Streptococcus</i> spp (7)	6	85.7
	CoNS (4)	1	25
	<i>Enterococcus</i> spp (3)	2	66.7
		40	51.3
GNB (N = 78)	<i>Eschericia coli</i> (33)	16	48.5
	<i>Klebsiella</i> spp (21)	11	52.4
	<i>Proteus mirabilis</i> (9)	5	55.6
	<i>P. aeruginosa</i> (8)	3	37.5
	<i>E. aerogenes</i> (4)	3	75
	<i>M. morganii</i> (3)	4	66.7
TOTAL (131)		73	55.7

3.4. Outcomes of Treatment of Cases of PPRM

Of the 120 women admitted for PPRM, 83 (69.1%) were discharged alive and alive, 14 (11.7%) were discharged against medical advice while 23 (19.2%) developed puerperal sepsis among who two (8.7%) died of overwhelming infection. Of the 124 babies delivered by mothers with PPRM, 106 (85.5%) were alive and well at the time of discharge, 8 (6.5%) were discharged against medical advice and 10 (8.1%) died. Thirty-four (28.6%) of the total 119 babies delivered alive developed clinical sepsis and 23 (19.3%) were culture positive cases. Fifty percent (n = 5) of the perinatal losses occurred at delivery due to low birth weight and prematurity (**Table 7**) while four (40%) of them had culture proven sepsis with MDR bacteria.

4. Discussion

Preterm prelabour rupture of fetal membranes (PPROM) is a significant cause of maternal and infant morbidity and mortality as well as infant mortality. It is the single most common identifiable factor associated with preterm delivery and it is one of the leading causes of neonatal admission into the newborn unit in this facility and many other centers in the country. Its globally incidence ranges from 0.7% - 3.5% [18], and it complicates 3% - 5% of pregnancies and causes up to 40% of all preterm deliveries [19]. Epidemiology of PPRM varies considerably from one geographical location to another with the prevalence higher in Africa than developed countries [20]. This study shows overall incidence of PPRM to be 4.4%, and this is comparable to reports of previous study in Kampala, Uganda which show incidence of 4.7% [21]. Okonofua *et al.* and Adewumi *et al.* earlier reported rates of 2.4% and 5.7% in this hospital [22] [23]. However, lower incidence rates of 2.5% and 3.3% were respectively reported in South-east Nigeria [24]

[25]. Rates in studies from other countries include South Africa (0.2%) [26], Ethiopia (1.4%) [27], Saudi Arabia (1.26%) [28], Bangladesh (2.7%) [29], Canada (2.3%) [30] and India (0.8% and 8.9%) [31]. This variability in incidences of PPROM is largely because of the multiple risk factors that have been associated with PPROM.

Table 7. Treatment outcomes of cases of PPROM.

Clinical Conditions		Frequency	Percentage
PPROM Outcome	Alive and well	83	69.1
	DAMA	14	11.7
	Had puerperal sepsis*	23	19.2
Puerperal Sepsis	Alive	21	91.3
	Dead	2	8.7
Neonatal Outcome	Alive	106	85.5
	DAMA	8	6.5
	Dead	10**	8.1
Culture Proven Neonatal Sepsis	Alive	19	82.6
	Dead	4	17.4

DAMA—Discharge against medical advice; *Two of the women who had puerperal sepsis died. **Four of the neonates died of sepsis caused by multidrug-resistant bacteria.

This study revealed a positive culture in 95 (79.2%) of the sample population with 131 bacteria isolates. This affirms the strong link between the occurrence of PPROM and genital tract infection. The positive culture rate in this study is similar to what has been previously reported in Nigeria [12] [13] [22]. Other studies from Togo, Uganda and Germany reported lower incidence rates of 48.3%, 30% and 41% respectively [21]–[24]. The high culture rate may be attributable to late presentation by patients as well as meticulous specimen transportation and processing protocols employed in this study.

Social demographic status also affirms a strong link between low parity and low socio-economic status [16] (mean parity, economic status and age of the patient). This agrees with Okonofua study in this center [15].

There is wide variation in bacterial isolates from cases of PPROM across regions and countries; majority of ours are Gram negative bacteria and this finding entirely agrees with a previous Nigerian study and study from Togo and India [17] [20] [22]. Several other studies however reported a preponderance of Gram positive bacteria [18] [20]–[23]. *Staphylococcus aureas* was the commonest organism isolated (39; 29.8%), and this is similar to findings by Musaba *et al.* in Uganda and Karat *et al.* in India [5] [9]. A systematic review of literature from Chinese data base involving 36 studies also reported *Staphylococcus aureas* as the predominant bacteria [18]. This however is in contrast to studies by Okonofua *et al.* and Adewumi *et al.* in this center [15] [22], Aboyeji *et al.* in Ilorin, Nigeria [32] and

others [8] [22] [24] who observed numerical dominance of other bacteria. The exact reason for the contrasting report is not known, our study also noted *Escherichia coli* as the most common organism of its category, this Gram-negative aerobic organism has been found to be the leading bacteria implicated in PPRM in previous studies in India [8] [17]. These organisms penetrate fetal membrane to cause intra amniotic infection and subsequently lead to rupture of fetal membranes [21]. Wide varieties of bacterial isolates both Gram positive cocci and Gram-negative bacilli in this study is similar to other authors who also reported a wide variety of aerobic and anaerobic bacterial isolates [22] [26]. These organisms may be part of wide variety of microbial isolates associated with PPRM as demonstrated [20]. *Streptococcus* spp found in our study have also been implicated in some other African studies as part of organism involved in PPRM [16] [18] [24]. The predominance of Gram-negative bacteria in this study may be because they are the major aerobic flora of the vagina which normally ascend into the amniotic sac should there be rupture.

The antibiotic resistance pattern of the microbial isolates in this study shows that ampicillin had the highest resistance pattern to both the Gram-negative bacilli and Gram-positive cocci (80% and 71% respectively). This is followed by cotrimoxazole having 73.6% and 77.1% for both Gram-positive and Gram-negative bacterial respectively. Berger *et al.*, Nimer *et al.*, and Okonofua *et al.*, also reported similar high resistance rates among bacteria to ampicillin and co-trimoxazole [8] [20] [22]. Other antibiotics that showed remarkable resistance include, amoxicillin, cloxacillin and chloramphenicol, and this susceptibility pattern were reported by several other authors [12] [15] [20]. Resistance rates were better for amoxiclav (37.7%), gentamycin (35.8%), cefotaxime (34.3%), ceftriaxone (37.7%), cefuroxime (34%) and erythromycin (34.0%). Bacterial resistance to erythromycin in our study is comparable to what was reported in Uganda [15]. Aboyeji *et al.* however reported low resistance rates to erythromycin (11.1%) and cefuroxime (9.3%) in a study conducted in the same country 15 years ago [32]. Bacterial isolates in this study showed justifiably good susceptibility to piperacilin-tazobactam, amikacin and imipenem, which are reserved drugs that are not frequently prescribed in our environment, and this is in agreement with a report from an Indian study by Berger *et al.* [8].

The findings of this study should be interpreted in the light of the following limitations: firstly, the specimens were collected after membrane rupture. The isolates may reflect ascending or contaminating genital tract flora. Also, the study design employed cannot establish infection as the cause of PPRM.

5. Conclusion

Most of the organisms cultured in this study were multiple drug resistant *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella species* of bacteria and may have potential to cause maternal and neonatal infections. They are sensitive to piperacillin-tazobactam, amikacin, meropenem, and cefuroxime. This evidence may

support the need for review of antibiotic treatment protocols for management of PPROM. Cefuroxime and erythromycin therefore remain good first line empiric antibiotics of choice for treatment of patients with PPROM.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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