Cervical amniotic fluid bacterial colonization, antibiotic susceptibility and associated factors among women with premature rupture of membranes at Mbarara Regional Referral Hospital, Southwestern Uganda

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Abstract

Background

Bacterial colonization is a recognized cause of premature rupture of membranes (PROM), a predictor of poor maternal and fetal outcomes. Despite routine use of antibiotics in women with PROM, data on antibiotic susceptibility patterns in Uganda are limited. We determined the prevalence and associated factors of cervical amniotic fluid bacterial colonization, and described the bacterial isolates and antibiotic susceptibility patterns among women seeking care at Mbarara Regional Referral Hospital (MRRH) in Southwestern Uganda.

Methods

We conducted a cross-sectional study at MRRH from December 21, 2020 to June 12, 2021. We consecutively enrolled women with PROM at ≥ 24 weeks of gestation, and aseptically picked two endo-cervical swabs irrespective of prior antibiotic use. Aerobic cultures were performed on blood, chocolate, MacConkey agars and incubated at 35–37◦C for 24–72 hours. Polymerase chain reaction (PCR) was performed on culture-negative samples. Antibiotic susceptibility was performed via Kirby-Bauer disk diffusion and dilution method. Interviewer-administered questionnaires were used to obtain participants' characteristics. We performed multivariable logistic regression to determine factors associated with bacterial colonization.

Results

We enrolled 144 participants with mean age of 26.5 ± 6.2 years. Prevalence of cervical amniotic bacterial colonisation was 35.4% (n = 51; 95%CI: 28.0–43.7). Six bacteria were isolated: *Klebsiella pneumoniae* (n = 15; 34.1%), *Staphylococcus aureus* (n = 11; 25.0%), *Enterobacter agglomerans* (n = 10; 22.7%), *Escherichia coli* (n = 3; 6.8%), *Streptococcus spp* (n = 3; 6.8%), and *Enterococcus faecalis* (n = 2; 4.6%). Ciprofloxacin exhibited the highest sensitivity (88.6%), followed by cefuroxime (75%), while all isolated bacteria demonstrated resistance to ampicillin. Factors independently associated with cervical amniotic fluid bacterial colonisation were prime gravidity (aOR = 2.69; 95%CI: 1.07–6.71, $p = 0.035$), obesity (aOR = 3.15; 95%CI: 1.10–9.11, $p = 0.024$) and being referred-in (aOR = 2.37; 95% CI: 1.04–5.3, $p = 0.038$).

Conclusion

Approximately one-third of the women had cervical amniotic fluid bacterial colonization, with all the bacterial isolates being resistant to ampicillin —the recommended first line of treatment for PROM by the Ministry of Health. There is a need to review the guidelines for the prophylactic use of ampicillin in PROM in our setting. Revising treatment protocols and considering alternative antibiotics based on local

resistance patterns could improve patient outcomes and prevent complications associated with ineffective antibiotic therapy in the context of PROM.

Background

Premature rupture of membranes (PROM) refers to the spontaneous disruption in the integrity of the amniotic sac with leakage of amniotic fluid after 24 weeks of gestation but before the onset of labor[1]. It is a common obstetric condition occurring in 10% of pregnancies globally and causing significant maternal and prenatal morbidity and mortality[2]. PROM is influenced by various factors, including bacterial invasion of the amniotic cavity, sterile inflammation, low body mass index, and maternal stress. Sterile inflammation plays a significant role in the weakening of the fetal membranes, involving the release of pro-inflammatory biomarkers, growth factors, and matrix-degrading enzymes by pattern recognition receptors (PRPs), thereby triggering an inflammatory response independent of bacterial presence[3] [4]. Ascending bacterial infection from the lower genital tract is the primary cause of preterm premature rupture of membranes (PROM), resulting in infection and inflammation of the fetal membranes and placenta. The prevalence of bacteria colonization in the cervical amniotic fluid varies between 20% and 50%, influenced by geographical region and the specific method employed to detect the presence of bacteria [5]. A study conducted at Mulago National Referral Hospital in Uganda revealed a prevalence of 30% for bacteria colonization in the cervical amniotic fluid[6].

Culture is considered the gold standard for determining bacterial colonization; however, some bacteria that have been implicated in the causation of PROM such as *Urea urealyticum, Mycoplasma species, Fusobacterium nucleatum, Leptotrichia species* are uncultivable or difficult to cultivate [7, 8]. Molecular-based identification techniques, such as the polymerase chain reaction (PCR), prove invaluable in cases where traditional cultivation methods are inadequate [9, 10]. Cervical amniotic fluid bacterial colonization in women with PROM has also been reported to be associated with several factors, including duration of PROM, prior antibiotic use, gestation age, obesity, urinary tract infections, and abnormal vaginal discharge [11, 12].

Group B streptococcus (GBS), a common bacterium found in the vagina and rectum of women, has long been associated with the causation or complication of most cases of preterm PROM [13, 14]. In response, Uganda's Ministry of Health adopted the World Health Organization's (WHO) recommendation of intravenous ampicillin along with oral erythromycin for the initial 48 hours, followed by oral amoxicillin or erythromycin for five days, as a prophylactic treatment for PROM[15]. However, recent studies conducted in Asia and Africa have indicated a shift in bacterial patterns in the amniotic fluid of women with PROM, with a decrease in GBS predominance and an increase in the prevalence of Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus; furthermore, there is growing evidence of antibiotic resistance among these bacteria in women with PROM[16, 17]. Data on bacterial isolates and antibiotic susceptibility patterns in Uganda, and particularly in southwestern Uganda are limited, yet antibiotics are routinely administered to mothers with PROM in Uganda. Thus, continued use of the current antibiotic guidelines in PROM, without periodically reviewing the antibiotic resistance profile, may not only increase

resistance through the emergence of multidrug resistant strains, but also in drug waste and complications for patients due to ineffective treatment. This study determined the prevalence and associated factors of cervical amniotic fluid bacterial colonization, and described the bacterial isolates and antibiotic susceptibility patterns among pregnant women seeking care at Mbarara Regional Referral Hospital (MRRH) in Southwestern Uganda.

Materials and Methods

Study design and setting

A cross-sectional study was conducted at the maternity ward of MRRH from December 21, 2020 to June 12, 2021. MRRH is a public facility and a teaching hospital for Mbarara University of Science and Technology (MUST) located in Southwestern Uganda, and serves as a referral centre for over ten districts attracting women with different pregnancy complications including PROM. The hospital has a high volume of clients, with maternity admitting 11,000 women annually. The hospital also has well-established departments of obstetrics, microbiology and paediatrics among others. The MRRH microbiology and molecular laboratory is a level three accredited laboratory and participates in external quality control conducted by the Uganda National Health Laboratory Services and the American College of Pathologists.

Study variables

The dependent variable was cervical amniotic fluid bacterial colonization. Independent variables were socio-demographic factors (age and referral status), obstetric characteristics *(*gestational age, gravidity, number of antenatal care visits, duration of drainage of liquor, antibiotic use since drainage started and features of clinical chorioamnionitis, fever, maternal tachycardia, fetal tachycardia, foul smelling liquor, abdominal tenderness), and medical factors (underlying medical illnesses, including HIV/AIDS, diabetes mellitus, urinary tract infections, abnormal vaginal discharge and obesity). We categorized gestational age as preterm (< 37 weeks) or term and beyond (≥ 37 weeks).

Inclusion and exclusion criteria

We included pregnant women admitted at ≥ 24 weeks of gestation with PROM during the study period (from December 21, 2020 to June 12, 2021). The diagnosis of PROM was made by the attending obstetrician. There were no exclusion criteria.

Sample size and sampling

Sample size estimation for this study was performed using Kish Leslie's formula for cross-sectional surveys (Kish, 1965). The assumptions considered were a presumed proportion of cervical amniotic fluid colonization at 0.3[6], a desired margin of error of 5% at a 95% confidence level, and a source population of 240 women. Through a review of maternity registers, it was determined that the hospital admits approximately 40 women with PROM per month, leading to an estimated source population of 240

participants over a six-month period. Considering a 10% non-response rate, the final calculated sample size was determined to be 144 women. The participants were selected using consecutive sampling.

Collection of data and endocervical samples

Each participant gave a written informed consent. We used an interviewer-administered pretested questionnaire to obtain data on participants' demographics, medical, and obstetric factors. Two sterile individually packed endocervical samples were then collected by rotating the swab through 360 degrees in the endocervical canal (except for every 10th participant where two other samples were picked for analysis in another laboratory for quality control) and were labelled with unique participant numbers.

Sample testing by culture

The endocervical sample was inoculated onto 5% sheep blood agar, MacConkey agar, Mannitol salt agar, and modified Thayer martin agar to isolate aerobic bacteria. The inoculated media was incubated at 37°C aerobically for 24–72 hours. Modified Thayer martin agar plates were incubated in a humidified atmosphere with 5% carbon dioxide. Identification of the cultured isolate was done by conventional phenotypic and biochemical methods which included; catalase, coagulase, DNA-ase for *staphylococcus aureus* (which produces positive catalase, coagulase, and DNA-ase tests) and urease, citrate utilization, oxidase, triple sugar iron for identification and differentiation of gram-negative bacilli.

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method. The medium for fastidious organisms was chocolate agar and incubated in carbon dioxide. For non-fastidious organisms, we used Muller Hinton Agar (MHA) incubated aerobically at 37◦C. The inoculum density required for susceptibility testing was 0.5% McFarland. The choice of antibiotic discs was based on the type of organism(s) cultured. The following antimicrobial agents were employed: ceftriaxone (30µg), ciprofloxacin (5µg), amoxicillin (10µg), oxacillin (10µg) and erythromycin (15µg), gentamycin (10µg), amoxicillin/clavulanate (10µg), cefixime (10µg), cefuroxime (10µg), azithromycin (10µg), doxycycline (10µg) were used for susceptibility testing

Sample testing by PCR

Presence of 16S rRNA gene (16SrDNA) was established by PCR amplification of genomic DNA using the following set of primers: Forward (AGAGTTTGATCMTGGCTCAG) and reverse (GGACTACCAGGGTATCTAATCCTGTT) primers, that amplify the 16S rRNA gene, that were added to the 2 times (2X) master mix containing standard buffer, dNTPs and Taq polymerase (M0486S) and nuclease-free water as follows: 12.5 µL of the 2X master mix; 1.0 µL forward (25 µM), 1.0 µL reverse (25 µM), 5 µL DNA template. RNAase-free-H_2O was used to make up to final reaction volume of 25µL.

PCR conditions were as follows: Initial denaturation at 95°C for 60 seconds, followed by 35 cycles at 95°C for 10 seconds, 54°C for 10 seconds, and 72°C for 50 seconds with a final extension of 72°C for 5 minutes (1, 2). Gel electrophoresis was performed using a 1.2% agarose gel containing Safe View DNA stain, 6x loading dye (Thermo Scientific #R0611), and 500 bp molecular weight marker (NEB-Biolabs

#N3231L) for 45minutes at 120 v. PCR amplicons were visualized using the Gene-Flash Trans-illuminator as shown in Fig. 1.

Data management and analysis

Data were entered into Redcap and exported to STATA version 15 (StataCorp, Texas, USA) for analysis. The prevalence of cervical amniotic fluid bacterial colonization was determined by calculating the proportion of women who tested positive for either a positive culture or PCR, divided by the total number of women enrolled in the study, and expressed as a percentage.

The distribution of bacterial isolates was visually represented using a bar graph, where each isolate was depicted alongside its corresponding total count and percentage relative to the overall isolates. In terms of antibiotic susceptibility, a tabular format was utilized to present the susceptibility patterns of the isolates, indicating the frequencies and percentages for each bacterial isolate.

To identify factors associated with cervical amniotic fluid bacterial colonisation we used univariable and multivariable logistic regression. At univariable analysis, odds ratios and their corresponding 95% confidence intervals were reported. Variables with a p-value < 0.2 at univariable analysis and biologically-plausible factors (gestational age, durational of PROM, presence of urinary tract infection, history of abnormal vaginal discharge and presence of abdominal tenderness) were included in multivariable analysis model. Factors with p-value < 0.05 at multivariable analysis were taken as statistically significant.

Results

Baseline socio-demographic and obstetric characteristics of study participants

We enrolled 144 participants with mean age of 26. 5 ± 6.2 years; most participants (75%) were in the 20–30-year age category (Table 1). The women with and those without cervical amniotic fluid bacterial colonization did not differ significantly with regard to the distribution of most of the baseline characteristics except for gravidity (p = 0.009) and referral status (p = 0.038). Primigravidae women had a higher proportion of bacterial colonization (54.9%) compared to multigravidas (45.1%). A higher proportion of women who were referred to the hospital (70.6%) had cervical amniotic fluid bacterial colonization compared to those who were not referred (52.7%).

Table 1

Socio-demographic, obstetric and Clinical characteristics of women with premature rupture of membranes at Mbarara Regional Referral Hospital, December 2020–June 2021, (N = 144)

Variables		Bacterial colonisation		p-value
	Overall, N = 144(%)	Yes, N = 51(%)	No, N = 93 (%)	
Age (years)				
20–30	108 (75.0)	39 (76.5)	69 (74.1)	
<20	18 (12.5)	9 (17.6)	9 (9.7)	0.265
35+	18 (12.5)	3 (5.9)	15 (16.1)	0.117
Residence	88 (61.1)	33 (64.7)	55 (59.1)	0.513
Rural	56 (38.9)	18 (35.3)	38 (40.9)	
Urban				
Marital status				
Married	129 (89.6)	44 (86.7)	85 (91.4)	
Not married	15 (10.4)	7 (13.7)	8 (8.6)	0.340
Referred				
No	59 (41.0)	15 (29.4)	44 (47.3)	
Yes	85 (59.0)	36 (70.6)	55 (52.7)	0.038
Body mass index				
Normal	41 (28.7)	10 (19.6)	31 (33.7)	
Overweight	60 (42.0)	23 (45.1)	37 (40.2)	0.145
Obese	42 (29.4)	18 (35.3)	24 (26.1)	0.078
Urinary Tract Infection				
No	93 (64.6)	35 (68.6)	58 (62.4)	
Yes	51 (35.4)	16 (31.3)	35 (37.6)	0.453
Abnormal Vaginal Discharge				
No	131 (91.0)	45 (88.2)	86 (92.5)	
Yes	13 (9.0)	6 (11.8)	7 (7.5)	0.400

SD: Standard Deviation; PROM: Premature rupture of membranes

Variables		Bacterial colonisation		p-value
	Overall, N = 144(%)	Yes, N = 51(%)	No, N = 93 (%)	
HIV status				
Negative	127 (88.2)	46 (90.2)	81 (87.1)	
Positive	17 (11.8)	5 (9.8)	12 (12.9)	0.583
Gravidity				
Multigravida	86 (59.7)	23 (45.1)	63 (67.7)	
Primigravida	58 (40.3)	28 (54.9)	30 (32.3)	0.009
Gestational age				
<37 weeks	53 (36.8)	17 (23.3)	36 (38.7)	
≥37 weeks	91 (63.2)	34 (66.7)	57 (61.3)	0.523
ANC attendance				
≤4	87 (60.4)	30 (58.8)	57 (61.3)	
>4	57 (39.6)	21 (41.2)	36 (38.7)	0.772
Duration of PROM				
<12 hours	84 (58.3)	27 (53.0)	57 (61.3)	
≥ 12hours	60 (41.7)	24 (47.1)	36 (38.7)	0.332
Presence of foul-smelling liquor				
No	121 (84.0)	40 (78.4)	81 (87.1)	
Yes	23 (16.0)	11 (21.6)	12 (12.9)	0.179
Abdominal tenderness				
No	137 (95.1)	49 (96.1)	88 (94.6)	
Yes	7 (4.9)	2 (3.9)	5 (5.4)	0.699
Fever				
No	139 (96.5))	50 (98.0)	89 (95.7)	
Yes	5 (3.5)	1 (2.0)	4 (4.3)	0.474
Maternal tachycardia				

SD: Standard Deviation; PROM: Premature rupture of membranes

Variables		Bacterial colonisation		p-value
	Overall, N = 144(%)	Yes, N = 51(%)	No, N = 93 (%)	
No	115 (79.9)	43 (84.3)	72 (77.4)	
Yes	29 (20.1)	8 (15.7)	21 (22.6)	0.326
Antibiotic use since draining started				
Yes	29 (20.1)	12 (23.5)	17 (18.3)	
No	115 (79.9)	39 (76.5)	76 (81.7)	0.454
SD: Standard Deviation; PROM: Premature rupture of membranes				

Prevalence of cervical amniotic fluid bacterial colonization

Among the 144 women with PROM at gestational age ≥ 24 weeks, a total of 51 participants were found to have bacterial colonization, resulting in a prevalence of 35.4% (95% confidence interval [CI]: 28.0-43.7).

Bacterial isolates from cervical amniotic fluid

A total of six different bacteria were isolated, with each sample containing only one bacterial isolate. Among the 44 isolates, the majority (n = 28; 63.6%) were Gram-negative. *Klebsiella pneumoniae* was the most frequent isolate (n = 15; 34.1%), followed by *Staphylococcus aureus* (n = 1125.0%), *Enterobacter agglomerans* (n = 10; 22.7%), and the remaining isolates consisted of *Escherichia coli* (6.8%), *Streptococcus spp* (6.8%) and *Enterococcus faecalis* (4.6%) (Fig. 2).

Antibiotic susceptibility patterns for the bacterial isolates

Of the 44 isolated bacteria, a notable majority were sensitive to ciprofloxacin (88.6%), cefuroxime (75%) and ceftriaxone (72.7%). However, all the isolated bacteria were resistant to ampicillin, while a significant portion were resistant to amoxycillin (72.7%) and azithromycin (54.5%) (Table 2).

Among all the isolates of *Klebsiella pneumoniae*, 100% showed resistance to ampicillin, while 86.7% (13/15) were resistant to amoxicillin. Fourteen out of fifteen isolates (93.3%) were sensitive to ciprofloxacin, cefuroxime, and gentamicin, and 60% (9/15) were sensitive to ceftriaxone.

For the 11 isolates of *Staphylococcus aureus*, all showed resistance to ampicillin, and 81.8% (9/11) were resistant to erythromycin. However, 90.9% (10/11) were sensitive to ceftriaxone and cefuroxime, and 81.8% (9/11) were sensitive to ciprofloxacin. All isolates of *Streptococcus spp* exhibited resistance to ampicillin and erythromycin. However, they showed sensitivity to azithromycin, cefuroxime, ceftriaxone, gentamicin, and ciprofloxacin (Table 2).

Table 2

Antibiotic susceptibility patterns of the bacterial isolates in cervical amniotic fluid of women with premature rupture of membranes at Mbarara Regional Referral Hospital, December 2020–June 2021, (n = 44)

Antibiotics		Bacterial Isolates						
		S. aureus (n = 11), n (%)	*Strep.* (n = 3), n (%)	*E. faecalis* (n = 2), n (%)	*Klebsiella* (n = 15), n (%)	*E. coli* (n = 3), n (%)	*E. agglom* (n = 10), n (%)	Total (n = 44), n (%)
AMO	S	5 (45.5)	1 (33.3)	0 (0.0)	2 (13.3)	1 (33.3)	3 (30.0)	12 (27.3)
	R	6 (54.6)	2 (66.7)	2 (100)	13 (86.7)	2 (66.7)	7 (70.0)	32 (72.7)
AMP	S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	R	11 (100)	3 (100)	2 (100)	15 (100)	3 (100)	10 (100)	44 (100)
AMOCLAV	S	9 (81.8)	2 (66.7)	0 (0.0)	9 (60.0)	1 (33.3)	5 (50.0)	26 (59.1)
	R	2 (18.2)	1 (33.3)	2 (100)	6 (40.0)	2 (66.7)	5 (50.0)	18 (40.9)
OXAC	S	8 (72.7)	2 (66.7)	2 (100)				12 (75.0)
	R	3 (27.3)	1 (33.3)	0 (0.0)				4 (25.0)
AZITHRO	S	5 (45.5)	3 (100)	1 (50.0)	8 (53.3)	1 (33.3)	2 (20.0)	20 (45.5)
	R	6 (54.5)	0 (0.0)	1 (50.0)	7 (46.7)	2 (66.7)	8 (80.0)	24 (54.5)
ERYTH	S	2 (18.2)	0 (0.0)	0 (0.0)				2 (12.5)
	R	9 (81.8)	3 (100)	2 (100.0)				14 (87.5)
CEFIX	S	8 (72.7)	2 (66.7)	0 (0.0)	6 (40.0)	1 (33.3)	8 (80.0)	25 (56.8)
	R	3 (27.3)	1 (33.3)	2 (100)	9 (60.0)	2 (66.7)	2 (20.0)	19 (43.2)
CEFTRI	S	10 (90.9)	3 (100)	1 (50.0)	9 (60.0)	2 (66.7)	7 (70.0)	32 (72.7)
	R	1 (9.1)	0 (0.0)	1 (50.0)	6 (40.0)	1 (33.3)	3 (30.0)	12 (27.3)

Antibiotics		Bacterial Isolates						
		S. aureus (n = 11), n (%)	*Strep.* (n = 3), n (%)	*E. faecalis* (n = 2), n (%)	*Klebsiella* (n = 15), n (%)	*E. coli* (n = 3), n (%)	*E. agglom* (n = 10), n (%)	Total (n = 44), n (%)
CEFUROX	S	10 (90.9)	3 (100)	2 (100)	10 (66.7)	2 (66.7)	6 (60.0)	33 (75.0)
	R	1 (9.1)	0 (0.0)	0 (0.0)	5 (33.3)	1 (33.3)	4 (40.0)	11 (25.0)
CIPRO	S	9 (81.8)	3 (100)	2 (100)	14 (93.3)	3 (100)	8 (80.0)	39 (88.6)
	R	2 (18.2)	0 (0.0)	0 (0.0)	1 (7.1)	0 (0.0)	2 (20.0)	5 (11.4)
GENTA	S	6 (54.5))	3 (100)	1 (50.0)	10 (66.7)	1 (33.3)	6 (60.0)	27 (61.4))
	R	5 (45.5)	0 (0.0)	1 (50.0)	5 (33.3)	2 (66.7)	4 (40.0)	1 7 (38.6)
DOXY	S	0 (0.0)	1 (33.3)	0 (0.0)	5 (33.3)	0 (0.0)	4 (40.0)	10 (22.7)
	R	11 (100)	2 (66.7)	2 (100)	10 (66.7)	3 (100)	6 (60.0)	34 (77.3)

AMP: ampicillin, *AMO*: amoxicillin, *AMOCLAV*: amoxicillin/Clavulanic acid, *AZITHRO*: azithromycin, *OXAC*: oxacillin, *CEFIX*: cefixime, *CEFTRI*: ceftriaxone, *CIPRO*: ciprofloxacin, *DOXY*: doxycycline, *GENTA*: Gentamycin, *CEFUROX*: cefuroxime, *ERYTH*: erythromycin, *S. aureus*: *Staphylococcus aureus*, *E. coli: Escherichia coli, E. agglom: Enterobacter agglomerans, E. faecalis: Enterococcus faecalis*, S: Sensitive, R: Resistant

Factors associated with cervical amniotic fluid bacterial colonization

Being a primigravida, obese or being referred were associated with bacterial colonization. Primigravidae were about 2.7 times more likely to have cervical amniotic fluid bacteria colonisation as compared to multigravidas (aOR: 2.69, 95% C.I: (1.07–6.71), $p = 0.035$). Obese women had three times higher odds of cervical amniotic fluid bacteria colonisation compared to those with a normal body mass index (aOR: 3.15, 95% C.I: (1.10–9.11), $p = 0.024$). Women who were referred had approximately 2.37 times higher odds of cervical amniotic fluid bacterial colonization compared to those who were not referred (aOR = 2.37, 95%CI: 1.04–5.3, p = 0.038) (Table 3).

Table 3
Factors associated with cervical amniotic fluid bacterial colonisation among women with PROM at Mbarara Regional Referral Hospital, December 2020–June 2021

Variables	bacterial colonization (n = 51), n (%)	cOR (95% C.I)	p-value	aOR (95% C.I)	p-value
Age (years)					
20–34	39 (76.5)	Ref.		Ref.	
<20	9 (17.6)	1.77 (0.65–4.83)	0.265	1.05 (0.24–3.14)	0.931
35+	3 (5.9)	0.35 (0.10–1.30)	0.117	0.48 (0.12–1.93)	0.298
Gravidity					
Multigravida	23 (45.1)	Ref.		Ref.	
Primigravida	28 (54.9)	2.56 (1.27–5.16)	0.009	2.69 (1.07–6.71)	0.035
Body mass index					
Normal	10 (19.6)	Ref.		Ref.	
Overweight	23 (45.1)	1.93 (0.80–4.66)	0.145	1.62 (0.62–4.30)	0.323
Obese (≥ 30)	18 (35.3)	2.33 (0.91–5.95)	0.078	3.15 (1.10–9.11)	0.034
Gestational age					
≥37 weeks	17 (23.3)	Ref.		Ref.	
<37 weeks	34 (66.7)	0.79 (0.39–1.62)	0.523	0.89 (0.39–2.01)	0.779
Duration of PROM					
<12 hours	27 (53.0)	Ref.		Ref.	
≥ 12hours	24 (47.1)	1.41 (0.71–2.81)	0.332	1.49 (0.68–3.26)	0.323
Presence of foul-smelling liquor					
No	40 (78.4)	Ref.		Ref.	

cOR: crude Odds Ratio; **Ref**: Reference group; **CI**: confidence Interval; **aOR**: adjusted Odds Ratio;

PROM: Premature rupture of membranes

Variables	bacterial colonization (n = 51), n (%)	cOR (95% C.I)	p-value	aOR (95% C.I)	p-value
Yes	11 (21.6)	1.86 (0.75–4.57)	0.179	2.05 (0.68–6.23)	0.204
Urinary tract infection					
No	35 (68.6)	Ref.		Ref.	
Yes	16 (31.3)	0.76 (0.37–1.56)	0.453	0.54 (0.24–1.23)	0.141
History of abnormal vaginal discharge					
No	45 (88.2)	Ref.		Ref.	
Yes	6 (11.8)	1.64 (0.52–5.17)	0.400	2.01 (0.56–7.95)	0.288
Referred					
No	15 (29.4)	Ref.		Ref.	
Yes	36 (70.6)	2.15 (1.04–4.46)	0.038	2.37 (1.04–5.37)	0.038
cOR: crude Odds Ratio; **Ref**: Reference group; **CI**: confidence Interval; **aOR**: adjusted Odds Ratio;					
PROM: Premature rupture of membranes					

Discussion

This study found that about one-third of women (35%) with PROM seeking care at MRRH had cervical amniotic bacterial colonization. The most common bacteria isolate was *Klebsiella pneumoniae* followed by *Staphylococcus aureus*. There was good sensitivity to quinolones and cephalosporins and marked resistance to penicillins. Cervical amniotic bacterial colonization was associated with being a prime gravida, being obese and being referred in. Overall these findings highlight the need to periodically review and update guidelines for the prophylactic use of antibiotics in PROM management; revising treatment protocols and considering alternative antibiotics based on local resistance patterns could improve patient outcomes and prevent complications associated with ineffective antibiotic therapy.

The prevalence of cervical amniotic bacterial colonization of 35% reported in the current study is consistent with findings from studies conducted at Mulago Hospital, Uganda (30% in 2017), and Wayne State University, USA (41% in 2015) [6] [5]. However, a study at Stanford University, USA in 2010 reported a higher prevalence of 50% [18]. The similarity of our findings with those at Wayne University could be attributed to the use of universal primers for PCR, which allows for the detection of a broad range of bacteria. In contrast, the higher prevalence at Stanford University may be due to the use of both universal

primers and group-specific primers, enabling the detection of bacterial presence in a larger number of samples.

The observed high prevalence of amniotic fluid bacterial colonization in our study is concerning, as previous research has linked such colonization to adverse pregnancy outcomes for both mothers and fetuses.[19]. For example, a study investigating the effects of amniotic fluid bacterial colonization on uterine activity and delivery outcomes found associations with poor cervical dilatation, response to oxytocin, and an increased risk of intrapartum infection [20]. Intrauterine infection following ascending vaginal colonization has also been implicated in causation of preterm labor, preterm births, still births, early onset neonatal sepsis among other complications[21].

Klebsiella pneumoniae, the commonest isolate in our study, has also been found to predominate amniotic fluid colonisation in PROM in studies conducted at a national referral hospital in Uganda, [6] and in Nigeria [17]. Gastrointestinal tract is a major reservoir of *Klebsiella pneumoniae* [22]. The close proximity of the gastrointestinal and genital tracts poses a high risk for contamination, allowing the bacteria to ascend to the cervix. This ascending colonization can lead to inflammation and subsequent rupture of the amniotic membrane[23]. Some strains of *K. pneumoniae* also lack the mannose content of the capsular polysaccharide that is recognized by the surface lectin of macrophages to mediate complement and antibody-independent phagocytosis. This makes them virulent and enables them to evade the body's defence mechanisms [23].

Staphylococcus aureus, a commonly found bacterium in the human skin microbiota, emerged as the second most prevalent isolate in our study. Similar findings have been reported in other studies conducted in India [24] and a meta-analysis from China [16], indicating its predominance in amniotic fluid colonization during PROM. As a resident flora on the skin, *Staphylococcus aureus* can easily migrate to the genital tract [25] and subsequently ascend to the cervix. This ascent can lead to infection and inflammation of the amniotic membranes, ultimately resulting in PROM. Moreover, in cases where PROM has already occurred, the presence of *Staphylococcus aureus* further exacerbates the risk of complications[23]. Of particular significance, *Staphylococcus aureus* produces α-toxin, which facilitates the formation of biofilms. Biofilm formation serves as a protective mechanism against dehydration and immune factors such as neutrophils and macrophages. This ability of *Staphylococcus aureus* to form biofilms enhances its survival and proliferation within the host environment [26, 27]

In contrast to studies conducted in Canada, Australia, and America where *Group B Streptococcus* (GBS) was identified as the most common organism colonizing amniotic fluid in PROM [28, 29], This discrepancy may be attributed to global variations in GBS colonization among pregnant women.A systematic review encompassing 85 countries revealed significant regional variation, with higher prevalence observed in America and Canada (25%) and lower incidence in East Africa (18%) [30]. These regional disparities can be influenced by factors such as temperature variations, genetic factors, and differences in population demographics[31]. Additionally, socioeconomic factors and variations in natural immunity within different populations can play a significant role.It is worth noting that a considerable

number of women in our study were referred from other healthcare facilities. As prophylaxis against GBS is part of the clinical guidelines[15], it is possible that these referred cases had already received prophylactic treatment, which could explain the lower prevalence of GBS in this study.

The bacteria isolated in our study exhibited significant resistance to the antibiotics recommended by the World Health Organization (WHO) and adopted by the Ministry of Health in Uganda for prophylaxis in PROM. All isolates were resistant to ampicillin, and the majority showed resistance to erythromycin, amoxicillin, and azithromycin. It is noteworthy that these guidelines were established based on recommendations from the ORACLE study, conducted over 20 years ago, which did not specifically focus on bacterial colonization of the female genital tract or antibiotic resistance [32]. The resistance to ampicillin observed in our study aligns with findings from other studies conducted in Nigeria, Tanzania, and Uganda [6, 17, 33]. This resistance pattern may be attributed to the overuse of these antibiotics, as penicillins are commonly prescribed for various bacterial infections [34]. The overuse of antibiotics directly contributes to the emergence of drug-resistant bacterial strains, and these resistance genes can be inherited or acquired and transferred among different species of bacteria. Additionally, antibiotics eliminate drug-sensitive competitors, providing a selective advantage for resistant bacteria to proliferate through natural selection[35]. The outdated nature of the guidelines, coupled with the alarming rates of resistance observed in this study, emphasize the importance of updating and tailoring treatment protocols to address the evolving antibiotic resistance landscape and improve patient outcomes.

The sensitivity of the isolated bacteria to certain cephalosporins was notable, with ceftriaxone demonstrating a sensitivity rate of 72.7%, and the less commonly prescribed second-generation cefuroxime exhibiting a sensitivity rate of 75%. Similar findings have been reported in a meta-analysis conducted in China, as well as studies conducted in Nigeria and Uganda [16] [17] [6]. This could be attributed to the stable β-lactam ring present in cephalosporins, which confers resistance to the action of beta-lactamases, enzymes that can inactivate certain antibiotics [36]. Overall, the highest sensitivity was observed for ciprofloxacin, at 88.6%, which aligns with findings from Nigeria where it reached 96.3% [17]. This could be attributed to ciprofloxacin having experienced a period of high resistance in the past, leading to its exclusion from many treatment protocols. As a result, its usage has been limited in recent years[37]. These findings further highlight the importance of selecting appropriate antibiotics for management of PROM based on their sensitivity profiles and considering the local resistance patterns. Continued surveillance of antimicrobial resistance could inform prescribing practices and ensure effective treatment outcomes.

Primigravidae were more likely to have cervical amniotic fluid bacteria colonisation as compared to multigravidas. Primigravidae may be at a greater risk of bacterial colonization than multigravidas due to their relatively limited interactions with the healthcare system and potential lack of exposure to medications that reduce bacterial colonization [38]. Given this increased vulnerability, primigravidae should be prioritized in the healthcare settings to avoid consequences associated with cervical amniotic fluid bacterial colonization in PROM.

Our findings revealed that obese women were more likely to exhibit cervical amniotic fluid bacterial colonization compared to women with a normal BMI. This observation aligns with previous studies that have demonstrated a link between obesity and bacterial colonization in the female genital tract[39, 40]. Notably, a significant proportion of bacteria colonizing the amniotic fluid in cases of PROM ascend from the genital tract[41]. The association between obesity and increased bacterial colonization in the genital tract may be attributed to several factors. Firstly, obesity can lead to poor genital hygiene due to excessive sweating and genital perspiration, creating an environment conducive to bacterial growth[40]. Furthermore, obese women often have higher estrogen levels resulting from peripheral aromatization, which promotes the maturation, proliferation, and accumulation of glycogen in vaginal epithelial cells. This glycogen serves as a favorable culture medium for bacterial growth[42]. As reported elsewhere [43, 44], it is crucial to prioritize the care of obese mothers by promoting good hygiene practices and implementing dietary and physical activity adjustments to mitigate the risk of cervical amniotic fluid bacterial colonization in cases of PROM.

Women who were referred in had higher odds of having cervical amniotic fluid bacteriology than those who were not referred. One plausible explanation for this observation is that a significant proportion of women with PROM in our study were referred from lower-level healthcare facilities where protocols for the accurate diagnosis and management of PROM may be lacking. Consequently, these mothers may have undergone multiple vaginal examinations and experienced a prolonged latency period before their presentation, primarily due to the challenges associated with referral transportation in our setting [45, 46]. However, the effect of the number of vaginal examinations was not studied in this study. This may have resulted into this residual confounding from this unstudied factor. Future studies should examine the specific factors related to referral and their impact on colonization risk in order to improve diagnosis and management strategies for this subgroup of women.

Limitations

Our study had some limitations that should be considered. Firstly, we were unable to conduct gene sequencing on the PCR-positive samples, which restricted our ability to identify and characterize specific bacterial isolates present in the samples. This information could have provided valuable insights into the microbial composition and potential virulence factors associated with cervical amniotic fluid bacterial colonization. Secondly, our study was conducted at a single site, which may limit the generalizability of our findings to other healthcare settings. The characteristics and practices at our study site may not fully represent the diverse populations and variations in bacterial colonization rates observed in different regions. Additionally, the lack of participant follow-up in our study prevented us from assessing important outcomes and implications related to bacterial colonization, such as maternal sepsis and neonatal infections. Future studies with longer follow-up periods are needed to evaluate the clinical outcomes and risks associated with cervical amniotic fluid bacterial colonization.

Despite these limitations, our study benefited from the use of highly sensitive PCR diagnostic testing, which improved the accuracy of detecting bacterial colonization in addition to traditional culture

methods. This enhanced sensitivity strengthens the validity of our findings regarding the prevalence of bacterial colonization in women with PROM.

Conclusions

Approximately one-third of the women in this study had cervical amniotic fluid bacterial colonization. Alarmingly, all the bacterial isolates demonstrated resistance to ampicillin, the recommended first-line treatment according to the Ministry of Health guidelines for PROM. This the high prevalence of bacterial colonization, coupled with the resistance patterns observed, underscores the urgency of revisiting the current guidelines for the prophylactic use of ampicillin in PROM in our setting. Future longitudinal studies should assess impact of cervical amniotic fluid bacterial colonization on maternal and perinatal outcomes, so as to develop evidence-based management strategies that optimize clinical outcomes of women with PROM in the region and similar low-resource settings.

Abbreviations

aOR: Adjusted Odds Ratio, **CI:** Confidence Interval, **cOR:** Crude Odds Ratio, **AIDS:** Acquired Immunodeficiency Syndrome, **ANC**: Antenatal care, **DNA**: deoxyribonucleic acid, **HIV**: Human Immunodeficiency Virus, **GBS**: Group B Streptococcus, **MRRH**: Mbarara Regional Referral Hospital, **PCR**: Polymerase Chain Reaction, **PROM**: Premature Rapture of Membranes, **rDNA**: ribosomal deoxyribonucleic acid, **RNA**: Ribonucleic acid, ***spp***: species, **UTI**: urinary tract infection.

Declarations

Ethics approval and consent to participate

This study was approved by the Faculty of Medicine Research Committee, Mbarara University of Science and Technology Research Ethics Committee (REC No. 10/11-20), and Uganda National council of Science and Technology (Ref. No. HS1459ES). Administrative clearance was obtained from the office of the Hospital Director, Mbarara Regional Referral Hospital, prior to conducting the study. The study complied with the Declaration of Helsinki. Written informed consent was obtained from each study participant before recruitment and participation in the study. Confidentiality of the study participants was ensured by using unique identifiers. Laboratory results were shared with the participants and the clinical care team for appropriate management.

Availability of data and materials

The datasets generated and analysed for this study are available from the corresponding author, upon request.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests with regard to publication of this work.

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Authors' contributions

All authors (BA, JN, JB, JM, AO, RM, DCA, GRM, MK and LT) made substantial contribution to the conception, design of the study, data analysis and interpretation. All the authors took part in drafting this manuscript and critically reviewed it and agreed to submit to this journal. They also gave the final approval of this version to be published and agreed to be accountable for all the aspects of the work.

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Figures

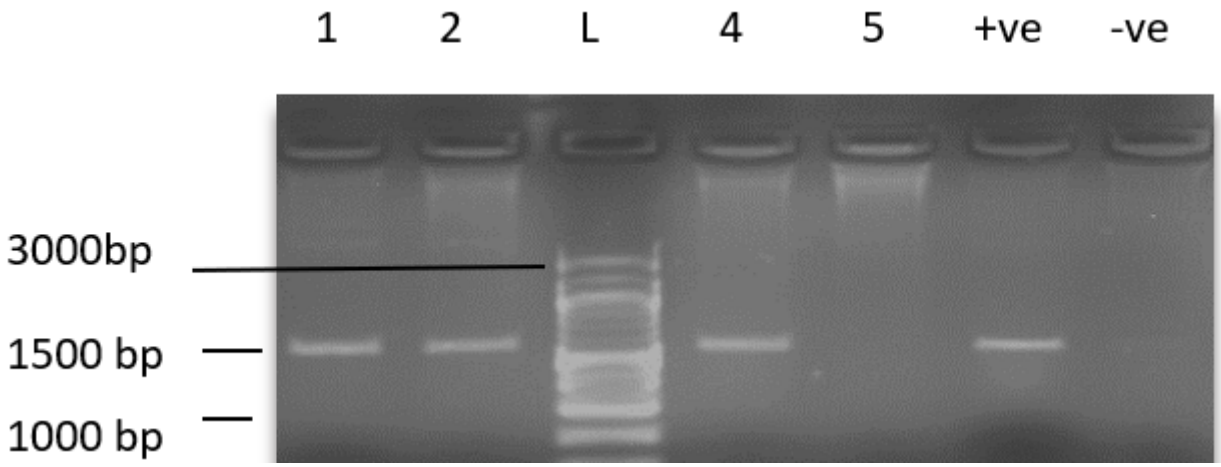

Figure 1

PCR results; An image of the PCR results; a horizontal band means presence of 16S rRNA, L is the control showing the molecular weight markers. Therefore; 1,2 & 4 are positive for 16S rRNA (gDNA) while 5 is negative (no amplification/band).

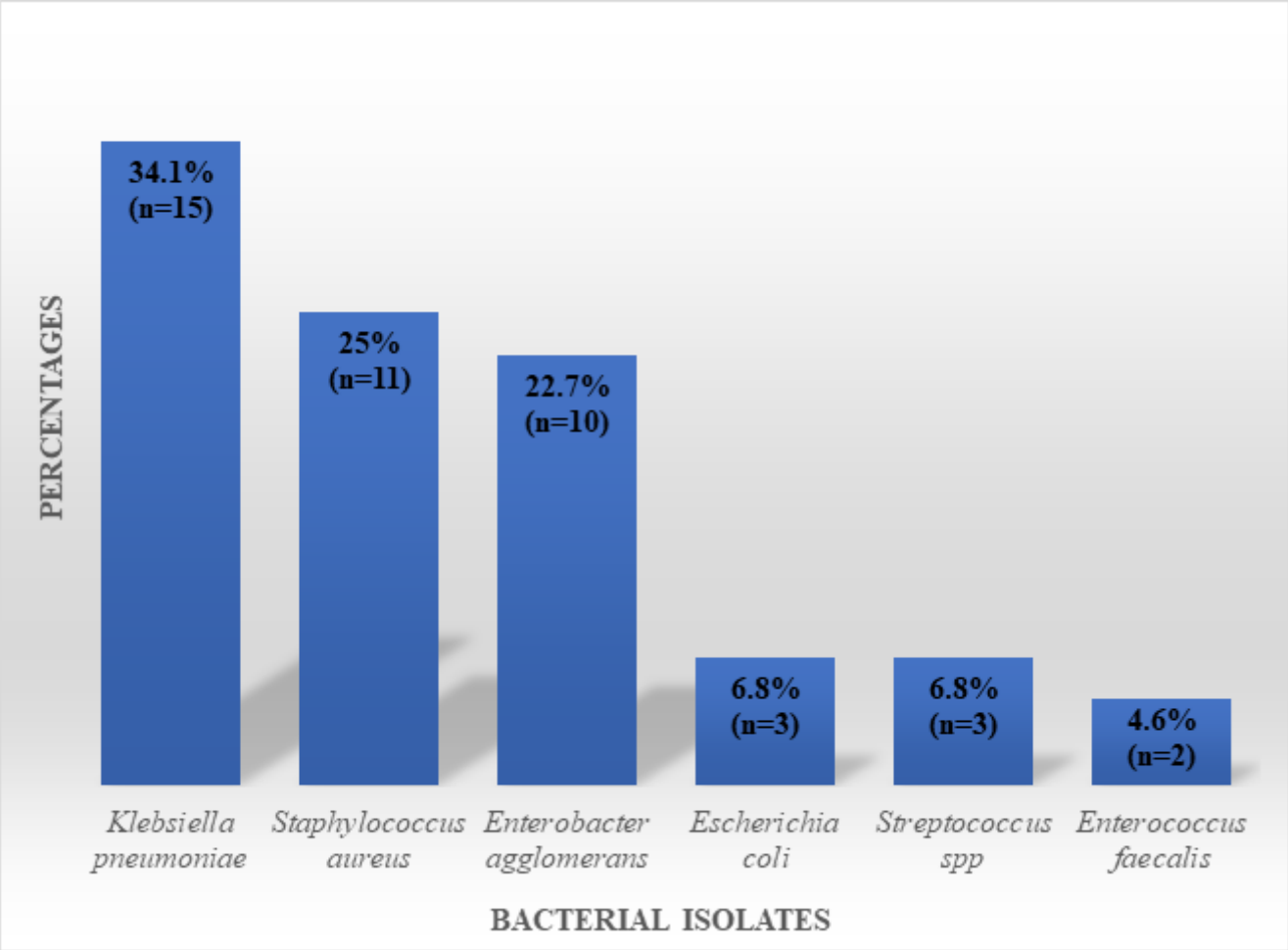

Figure 2

Bacterial isolates by culture in cervical amniotic fluid of women with premature rupture of membranes at Mbarara Regional Referral Hospital, December 2020–June 2021 (n=44)