Microorganisms associated with intraamniotic infection among women with preterm birth at Ruhengeri Referral Hospital, Rwanda: A case-control study

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Abstract

Background

Preterm birth could be a worldwide open well-being danger. It was assessed that each year 15 million neonates are born prematurely around the world, and 40% brought about from intrauterine infections.

Methods

A total of 40 women were selected. Of the 40 women, 20 had a premature birth, and the remaining 20 had a full-term birth. 120 Swab samples were collected from the placenta, amniotic fluids, and fetal membrane immediately after birth. The sterile cotton swab was used to collect samples and put into swabs Stuart plastic to avoid sample contamination. Samples were transported to the clinical microbiology laboratory at INES Ruhengeri for microbiological investigation. Gram staining, culture, and biochemical tests were performed. The independent t-test was used to test for significant difference between means of the two groups, while the chi-square test (x^2) was used to test for significant association with microorganisms and intraamniotic infections.

Results

The findings revealed that half of the participants were in the age range of 24–29 years. *Non- Albicans Candida* (32.7%) and mold (27.9%) were the foremost overwhelming confined microorganisms. Some microorganisms were common to the placenta, amniotic fluid, and fetal membrane. Only *Non-Albicans Candida* and *mold* were common to samples of both preterm and full-term women, *Staphylococcus species* was observed in placental and fetal membrane samples and absented in amniotic fluid. *Escherichia coli, Klebsiella species, Streptococcus species*, and *Candida Albicans* were only observed among women with preterm birth. To compare isolated microorganisms between both preterm and full-term birth, the significance test of mean was performed. There was a statistically significant difference between the two means in the amniotic fluid isolates (t = 4.023, P = 0.006522), placental membrane isolates (t = 7.17, P = 0.000372), and fetal membrane isolates (t = 6.7, P = 0.000537). Association with microorganisms and intraamniotic infection was statistically significant with *Escherichia coli* (x^2 = 3.98, P = 0.046044), *Streptococcus species* (x^2 = 5.53, P = 0.018693), *Yeast* (x^2 = 8.37, P = 0.003815) and *Candida Albicans* (x^2 = 3.98, P = 0.046044).

Conclusion

Invasion of the amniotic fluid, placenta, and fetal membranes by pathogenic microorganisms may be associated with the incidence of preterm labor and birth. Early diagnosis is recommended to avoid both maternal and fetal complications.

Background

Preterm birth is a birth that comes earlier than 37 weeks of the full gestation period. Existing data shows that every year 15 million babies are born prematurely around the world. Preterm delivery is the leading cause of neonatal death in the developed world [1]. The health consequences of preterm delivery commonly occur to neonatal periods but can be long-lasting across the life course. Spontaneous development of the fetus or medical intervention provided for pregnancy could be the leading cause of preterm birth to women [2]. The specific known factors of spontaneous preterm birth include infections, placental abruption, hormonal conditions, multiple gestations, etc. Intrauterine infection (intraamniotic infection) is the cause of around 40% of preterm deliveries globally [3]. It has been difficult to determine whether infections cause preterm delivery of women, but evidence shows that infection in the uterus could cause preterm birth. Inflammatory diseases on gestational tissues caused by infections are the major cause of preterm delivery [4]. This is evidenced by a relatively higher rate of microbial colonization and inflammatory conditions in the intraamniotic fluid of preterm labor patients compared to those lasting full term [5]

There is a lack of calcification on the rupture of membranes(ROM). The rupture of the membrane is a healthy sign of labor. Sometimes happens before onset of labor begins, it is considered as premature rupture of membrane(PROM) [6]. On the other hand, when the rupture of the membrane happens before 37 weeks, it is known as the preterm premature rupture of membrane(PPROM) which is a sign of intraamniotic infections in some cases [7]. Bacterial intrauterine infections are the major cause of preterm birth related infections. The amniotic cavity is considered to be free from infections, less than 1% of women in preterm labor have bacteria in their amniotic fluids [8]. It has been difficult to isolate bacteria of amniotic fluids before birth because of the risk posed by secondary complications from pregnancy. Most of the intraamniotic microbial colonization is not clinical and cannot be detected without microbiological analysis of amniotic fluid [9]. It has been estimated that 12.8% of positive amniotic fluid culture to occur to women with premature labor with an intact membrane. Some 22% of positive amniotic fluid was reported to women that had experienced premature labor with the intact membrane but who delivered preterm babies [10].

The rate of positive amniotic fluids was found to be 32.4% among women experiencing preterm premature rupture membranes(PPROM). During labor, more than 75% of these women suffer from the microbial invasion of the amniotic cavity (MIAC) due to the ruptured membrane which plays a protective role [11]. Women with microbial invasion of the amniotic cavity are at high risk to deliver a preterm baby, and to develop clinical chorioamnionitis [12].

Recent findings reported that the rate of preterm birth in Rwanda stood at 10%, despite efforts by the government of Rwanda to mitigate the same [13]. However, there is a lack of studies on intraamniotic-related infections and their influence on preterm birth. This study, therefore, investigated major microorganisms that contribute to intraamniotic fluid invasion among women with preterm birth at Ruhengeri referral Hospital.

Methods

Study setting

This study was carried out at Ruhengeri Referral Hospital located in Musanze District, Northern Province of Rwanda.

Study design

This was a case-control study. Data were collected from October 2018 to January 2019.

Study population and sample size

Cases consisted of women whose delivery was preterm and controls were selected from women with full-term delivery. Non-probability purposive sampling was used and 20 women for both preterm and term delivery were selected.

Sample processing and collection

To compare the presence of microorganisms and intraamniotic infection between cases and controls, swab samples were collected from amniotic fluid, placental, and fetal membrane. 3 samples were collected from each woman. Samples were collected immediately after delivery in the regional referral Hospital, Musanze. This was done using a sterile cotton swab and put in specimen bags (Stuart plastic) to avoid sample contamination. Samples were transported to INES Ruhengeri clinical microbiology laboratory for microbiological analysis. Microbiological analysis techniques including gram staining, culture, and biomedical tests were performed to isolate and identify microorganisms in amniotic fluid, placental membrane, and fetal membrane samples.

Laboratory techniques

Gram Staining

Gram stain technique was done to classify and differentiate the bacteria into Gram-Positive and Gram-Negative, microorganisms. After, smear preparation and application of different dyes, the slides were examined with the aid of light microscopy. Gram-positive microorganisms retain the primary dye Crystal violet and Gram-negative microorganisms take the color of the counterstain Safranin O.

Culture media preparation

The blood agar (Fluka®70133-500G) and MacConkey agar (QB-39-2707), culture media was used to isolate bacteria from the placental, fetal membrane, and amniotic fluid samples. This was done based on Gram stain results. The samples were inoculated into prepared plates with solidifying culture media, the inoculated plates were then incubated at 37 ˚C for 24 hours. Therefore, the plates with growth colonies were morphologically observed and examined for biochemical tests.

Biochemical Tests

Different biochemical tests were performed to differentiate bacterial species. The catalase and coagulase tests were used to differentiate *Staphylococcus* species and *Streptococcus* species gram-positive bacteria. For the identification of Gram-negative bacteria isolates different biochemical culture media were used to test different biochemical parameters. Simon's citrate agar was used to test the microorganism's ability to utilize citrate as a source of energy. Motility was confirmed when a wide filament like-form appeared in the SIM medium. An appearance of a red ring-like formed into its surface after adding in two to three drops of Kovac's reagent after 24 hours of incubation indicates indole positive. Urease positive was proved by a color change in pink for urea broth. Furthermore, Kligler iron agar (KIA) permitted differentiation of Gram-negative bacilli by their ability to ferment glucose or lactose was also used. Red color changes from yellow due to the PH indicator in response to acid production of the fermentation of the sugar.

Yeast and molds identification

Sabouraud Dextrose agar (TM 387/500 g) selective media for both yeast and molds was used. Prepared Sabouraud agar plates were inoculated by streaking, as with standard bacteriological media. Molds were typically incubated at room temperature (22 to 25 °C for 2 to 5 days) and yeasts were incubated at 37 °C for 24 h. The growing yeast colonies (creamy whitish) were confirmed by Germ tube to differentiate *Candida albicans* and *candida* other species, whereas, molds grew as filamentous colonies of various colors were identified morphologically and by using lactophenol cotton blue stain.

Statistical analysis

We analyzed the significant difference in the mean between the two groups and the association with isolated microorganisms and intraamniotic infection among women with preterm delivery. SPSS version 22 was used. Both t-test and chi-square tests were carried out to test for statistical associations. The level of significance was $\alpha = 0.05$.

Ethical consideration

The research ethics committee of INES Ruhengeri Institute of applied sciences provided a letter sanctioning the research to be conducted, but also an ethical approval letter was given by Ruhengeri Referral Hospital for swab sample collection. No names or any human identification data were recorded except the code number of the patient.

Results

Age distribution of the study participants

Table 1 shows the age distribution of the study population. 50% of participants in both preterm and full-term women were in the age range of 24-29years, and 20% aged 30–35 years. Pre-term women compared with full-term were aged 36–41 years. 15% compared with 10% and less likely to be aged 18–23 years.

Table 1
age distribution of the study participants

	Preterm women	Full term women	Total
Age group			
18–23	3(15%)	4(20%)	7(17.5%)
24–29	10(50%)	10(50%)	20(50%)
30–35	4(20%)	4(20%)	8(20%)
36–41	3(15%)	2(10%)	5(12.5%)
Total	20(100%)	20(100%)	40(100%)
Comparative profiles of microorganisms isolated from amniotic fluid			

Comparative profiles of microorganisms isolated from amniotic fluid

Table 2 shows the percentages of isolated microorganisms from amniotic fluid in both women with preterm and full-term birth. The predominantly isolated microorganisms among women with preterm birth were *mold* (28.8%) and *non-Albicans Candida (*28.8%). The *non-Albicans Candida* (66.7%) and *molds* (33.3%) were also predominant among women with full-term delivery in different proportions. No other microorganisms isolated from amniotic fluid were observed of women with full-term delivery. *Escherichia coli* (9.6%), *Klebsiella species* (3.8%), *Streptococcus species* (13.4%), *Staphylococcus species* (5.7%), and *Candida Albicans* (9.6%) were only isolated among women with preterm birth. The significance of the difference between the means of the two groups was done using a t-test. The difference between the means of the two groups was statistically significant (t = 4.023, P = 0.006522).

Table 2
Comparative profiles of microorganisms isolated from amniotic fluid

	Women with PTB	Women without PTB	Standard deviation	Independent t-test	df	P-value
Microorganism						
Escherichia coli	5(9.6%)	0(0.0%)				
Klebsiella species	2(3.8%)	0(0.0%)				
Streptococcus species	7(13.4%)	0(0.0%)				
Staphylococcus species	3(5.7%)	0(0.0%)				
Mould	15(28.8%)	2(33.3%)				
Non-albicans Candida	15(28.8%)	4(66.7%)				
Candida albicans	5(9.6%)	0(0.0%)				
total	52(100%)	6(100%)	4.0773	4.023	6	0.006522
$\sum$**D** = 46	**MD** = 6.5	$\sum$**(D-MD)**^{2}=99.75	**SE** = 1.541			
Comparison of microorganisms isolated from placental membrane samples						

Comparison of microorganisms isolated from placental membrane samples

Table 3 shows the percentages of isolated microorganisms from the placental membrane samples among women with preterm and full-term delivery. Isolated microorganisms from placental membrane taken from women with preterm birth were predominated by *mold* (25.8%) and *Yeast* (25.8%), the same condition was observed among women with full-term delivery where the predominant microorganisms were *non-Albicans Candida* (50%) and *mold* (30%). *Staphylococcus species* were also isolated from women with full-term birth and stood at 20%. Other microorganisms isolated from women with preterm delivery including *Staphylococcus species* (13.7%), *Streptococcus species* (12%), *Candida albicans* (8.6%), *Escherichia coli* (5.6%), and *Klebsiella species* (5.2%). These last microorganisms were uniquely observed among women with preterm birth excluding *Staphylococcus species.* The means difference (t = 7.17, P = 0.000372) of the two groups was statistically significant.

Table 3
Comparison of microorganisms isolated from placental membrane samples

	Women with PTB	Women without PTB	Standard deviation	Independent t-test	df	P-value
Microorganisms						
Escherichia coli	5(8.6%)	0(0.0%)				
Klebsiella species	3((5.2%)	0(0.0%)				
Streptococcus species	7(12%)	0(0.0%)				
Staphylococcus species	8(13.7%)	4(20%)				
mould	15(25.8%)	6(30%)				
Non-albicans Candida	15(25.8)	10(50%)				
Candida albicans	5(8.6%)	0(0.0%)				
total	58(100%)	20(100%)	1.988	7.17	6	0.000372
$\sum D = 38$	MD = 5.4	$\sum (D\text{-}MD)^2 = 23.72$	SE = 0.753			
Comparison of microorganisms isolated from fetal membrane samples						

Comparison of microorganisms isolated from fetal membrane samples

Table 4 reveals microorganisms isolated from fetal membrane samples of women with preterm and full-term delivery. *Mold* (26.3%) and *non-albicans Candida* (26.3%) were the isolated predominant microorganisms among women with preterm birth. These microorganisms also were predominant among women with term birth to 33.3% and 55.5% respectively. *Staphylococcus species* were observed among both preterm and full-term birth next to *mold* and *non-albicans Candida* with 14% and 11.1% respectively. *Streptococcus species* (12%), *Candida albicans* (8.7%), *Escherichia coli* (8.7%), and *Klebsiella species* (3.5%) were only observed among women with preterm delivery. The difference between the means of the two groups was statistically significant (t = 6.7, P = 0.000537).

Table 4
Comparison of microorganisms isolated from fetal membrane samples

	Women with PTB	Women without PTB	Standard deviation	Independent t-test	df	P-value
Microorganisms						
Escherichia coli	5(8.7%)	0(0.0%)				
Klebsiella species	2(3.5%)	0(0.0%)				
Streptococcus species	7(12.2%)	0(0.0%)				
Staphylococcus species	8(14%)	2(11.1%)				
mould	15(26.3%)	6(33.3%)				
Non-albicans Candida	15(26.3%)	10(55.5%)				
Candida albicans	5(8.7%)	0(0.0%)				
total	57(100%)	18(100%)	2.15	6.7	6	0.000537
$\sum D = 39$	MD = 5.5	$\sum (D\text{-}MD)^2 = 27.75$	SE = 0.814			
Microorganisms associated with intraamniotic infection						

Microorganisms associated with intraamniotic infection

Table5 shows microorganisms associated with intraamniotic infection among women with preterm delivery. Both samples from preterm and full-term birth were considered. This identified 7 microorganisms including *Escherichia coli, Klebsiella species, Streptococcus species, Staphylococcus species, mold, non-albicans Candida, and Candida albicans.* However, *Escherichia coli* (x^2=3.98, P= 0.046044), *Streptococcus species* (x^2=5.53, P=0.018693), *non-albicans Candida* (x^2=8.37, P=0.003815) and *Candida albicans* (x^2= 3.98, P=0.046044) were statistically significant. The overall association (x^2=24.084, P=0.000504) with all isolated microorganisms and intraamniotic infections was statistically significant.

Table5: Microorganisms associated with intraamniotic infection

	Women with PTB	Women without PTB	Total	Chi Square	df	P-value
Microorganisms						
Escherichia coli	15(11.8)	0(3.12)	15	3.98	1	0.046044
Klebsiella species	7(5.5)	0(1.4)	7	1.8	1	0.179712
Streptococcus species	21(16.6)	0(4.37)	21	5.53	1	0.018693
Staphylococcus species	19(19.7)	6(5.2)	25	0.14	1	0.708281
mould	45(46.6)	14(12.3)	59	0.284	1	0.594091
Non-albicans Candida	45(55)	24(14.3)	69	8.37	1	0.003815
Candida albicans	15(11.8)	0(3.12)	15	3.98	1	0.046044
Total	167	44	211	24.084	6	0.000504

Dataset						
Date of Sample collection	**Status**	**Samples names**	**ages**	**Placenta membrane**	**Fetal membrane**	**Amniotic fluid**
6/12/2018	Preterm birth	86	37	yeast, candida ssp.and staphylococcus ssp.	Yeast, candida ssp. And staphylococcus ssp.	Yeast, candida ssp. And staphylococcus ssp.
9/11/2018	Preterm birth	345	29	Candida albican.	Candida albican.	Candida albican.
6/12/2018	Preterm birth	82	38	Streptococcus ssp., yeast and candida albican	streptococcus ssp., yeast and candida albican	streptococcus ssp., yeast and candida albican
17/11/2018	Preterm birth	347	28	staphylococcus ssp.and yeast	staphylococcus ssp.and yeast	staphylococcus ssp.and yeast
18/11/2018	Preterm birth	357	25	staphylococcus ssp.and yeast	staphylococcus ssp.and yeast	staphylococcus ssp.and yeast
22/11/2018	Preterm birth	363	25	E.coli and yeast	E.coli and yeast	E.coli and yeast
20/11/2018	Preterm birth	378	27	streptococcus ssp., yeast and klebsiella ssp.	streptococcus ssp., yeast and klebsiella ssp.	streptococcus ssp., yeast and klebsiella ssp.
20/11/2018	Preterm birth	405	20	staphylococcus ssp., E.coli and yeast	Sterptococcus ssp.,E coli and yeast	Sterptococcus ssp.,E coli and yeast
22/11/2018	Preterm birth	435	28	Sterptococcus ssp.,E coli and yeast	Sterptococcus ssp., E coli and yeast	Sterptococcus ssp.,E coli and yeast
22/11/2018	Preterm birth	441	24	E.coli and yeast	E.coli and yeast	E.coli and yeast
23/11/2018	Preterm birth	468	28	streptococcus ssp. ,yeast and mould	streptococcus ssp. ,yeast and mould	streptococcus ssp. ,yeast and mould
23/11/2018	Preterm birth	473	31	E.coli and yeast	E.coli and yeast	E.coli and yeast
27/11/2018	Preterm birth	508	27	yeast and mould	yeast and mould	yeast and mould
28/112018	Preterm birth	589	39	staphylococcus ssp.	staphylococcus ssp.	staphylococcus ssp.
28/11/2018	Preterm birth	590	22	staphylococcus ssp.	staphylococcus ssp.	staphylococcus ssp.

26/11/2018	Preterm birth	509	32	No microbial growth	No microbial growth	No microbial growth
26/11/2018	Preterm birth	516	32	No microbial growth	No microbial growth	No microbial growth
26/11/2018	Preterm birth	518	20	E.coli, mould and yeast	E.coli, mould and yeast	E.coli, mould and yeast
30/11/2018	Preterm birth	651	25	streptococcus ssp.,yeast and mould	streptococcus ssp.,yeast and mould	streptococcus ssp.,yeast and mould
5/12/2018	Preterm birth	71	35	yeast and candida albican	yeast and candida albican	yeast and candida albican
10/12/2018	Full-term birth	165	32	yeast and mould	yeast and mould	No microbial growth
10/12/2018	Full-term birth	100	32	yeast and mould	yeast and mould	No microbial growth
12/12/2018	Full-term birth	185	25	staphylococcus ssp.and yeast	yeast	No microbial growth
12/12/2018	Full-term birth	92	24	No microbial growth	No microbial growth	No microbial growth
17/12/2018	Full-term birth	288	24	No microbial growth	No microbial growth	No microbial growth
17/12/2018	Full-term birth	166	25	staphylococcus ssp.and yeast	yeast	No microbial growth
17/12/2018	Full-term birth	283	33	No microbial growth	No microbial growth	No microbial growth
17/12/2018	Full-term birth	314	28	staphylococcus ssp.and yeast	staphylococcus ssp.and yeast	No microbial growth
9/12/2018	Full-term birth	148	22	No microbial growth	No microbial growth	No microbial growth
9/12/2018	Full-term birth	261	19	No microbial growth	No microbial growth	No microbial growth
18/12/2018	Full-term	307	25	yeast and mould	yeast and mould	yeast

	birth					
18/12/2018	Full-term birth	258	39	No microbial growth	No microbial growth	No microbial growth
14/12/2018	Full-term birth	236	39	No microbial growth	No microbial growth	No microbial growth
14/12/2018	Full-term birth	311	25	yeast and mould	yeast and mould	yeast
17/12/2018	Full-term birth	292	28	yeast and mould	yeast and mould	yeast and mould
17/12/2018	Full-term birth	295	28	yeast and mould	yeast and mould	yeast and mould
15/12/2018	Full-term birth	245	19	No microbial growth	No microbial growth	No microbial growth
15/12/2018	Full-term birth	171	22	No microbial growth	No microbial growth	No microbial growth
18/12/2018	Full-term birth	312	28	staphylococcus ssp.and yeast	staphylococcus ssp.and yeast	No microbial growth
18/12/2018	Full-term birth	287	33	No microbial growth	No microbial growth	No microbial growth

Discussion

Intrauterine infection is also known as Intraamniotic infection or clinical chorioamnionitis. It is a combination of infection in amniotic fluids, fetal membranes, and placental membranes [14]. This study analyzed the microbiological differences in amniotic fluid, placental membranes, and fetal membranes. *Escherichia coli, Streptococcus species, non-albicans Candida*, and *Candida albicans* were associated with intraamniotic infections and were observed in all three types of samples (Table 2,3&4). The study on the intraamniotic infection reported that isolated microorganisms from amniotic fluid samples were the normal flora of the vagina including *Escherichia coli* and *Streptococcus species* [15]. The ascendance of vaginal flora to the uterus can cause intrauterine infection which is a precursor to preterm birth and fetal deaths. A similar finding from a systematic review of candida chorioamnionitis among pregnant women revealed a high prevalence of *Candida albicans* among candida chorioamnionitis patients [16]. *Candida albicans* is the leading cause of most vaginal yeast infection among women. Its ascendancy to the uterus could lead to preterm delivery, birth impairment, and fetal death. Some microorganisms were

isolated from women with preterm birth and excluded from women that had carried their pregnancy to a full-term birth. Similar findings reported that *Escherichia coli* and *Streptococcus species* were isolated from women with intraamniotic infections [17]. The current study compared the microbial differences between women with preterm and women with full-term delivery. The mean difference between the two samples was statistically significant (Table 2,3, &4). The findings reported by Doyle et al showing microbial differences between preterm and term women. In their findings, *Fusobacterium*, *Streptococcus*, *Mycoplasma*, *Aerococcus*, *Gardnerella*, *Ureaplasma*, and *Enterobacteriaceae* were reported at a high percentage among preterm women but were absent in samples taken from women who had undergone full-term delivery [18]. The findings on preterm prelabour amniorrhexis and intraamniotic infection reported *Streptococcus agalactiae and Streptococcus milleri Lactobacillus sp, Enterobacter sp, Candida albicans, Streptococcus viridans, Streptococcus sanguis, Haemophilus influenza, and Staphylococcus epidermidis.* These microorganisms were isolated from fetal blood samples while *Enterobacter, C. Albicans, H. influenza*, *S. agalactia*, and *S. epidermidis* were isolated from amniotic fluid samples [19]. We isolated some similar microorganisms in both fetal membranes and amniotic fluid samples. We did not sample fetal blood samples. Tests of association with isolated microorganisms and intraamniotic infection were conducted, identified a statistical association of Escherichia *coli, Streptococcus species, Yeast*, and *Candida albicans* (Table 5). A similar finding reported different isolates but showed similarities by having isolates similar to what the present study isolated and this included S. *agalactiae, E. coli*, and *Staphylococcus coagulase-negative* [20]. Peng et al indicate the importance of these biomarkers in making a diagnosis for intrauterine infection or chorioamnionitis and instituting antibiotic management early to prevent preterm birth. However, the possibility of an antepartum diagnosis of these infections may be a challenge given the invasiveness of amniocentesis for obtaining an amniotic fluid sample [21]. These findings did not study which microbe was associated with intraamniotic infection among women with preterm labor and which organisms were associated with maternal or neonatal inflammatory symptoms such as fever. *Molds, Klebsiella, and Staphylococcus spp* were not associated with intraamniotic infection and past studies did not report any of them to contribute to clinical chorioamnionitis.

Conclusion

The invasion of microorganisms towards the uterus could lead to intraamniotic infection or clinical chorioamnionitis among pregnant women, and this could lead to preterm birth. There was a difference in microbial fauna among preterm and full-term birth regimes among women in all placental membranes, amniotic fluid, and fetal membrane samples. The mean difference between the two groups was statistically significant. *Escherichia coli*, *Streptococcus species*, *non-albicans Candida*, and *Candida albicans* were the major cause of intraamniotic infection or clinical chorioamnionitis among women with preterm birth. Although intraamniotic infections may be subtle, pregnant women should seek immediate medical care when they show signs such as pre-labor draining of fluids or are at higher or risk of intraamniotic infections.

Abbreviations

PTB
preterm birth
ROM
Rupture of membrane
PROM
Premature rupture of membrane
PPROM
Preterm premature rupture of membrane
MIAC
Microbial invasion of the amniotic cavity

Declarations

Ethics approval and consent to participate

INES Ruhengeri Institute of applied sciences research committee provided an ethical letter for this study to be carried out, but also Ruhengeri Referral hospital provided ethical approval for sample collection in a maternity setting. Samples were obtained externally from the mothers, and the verbal informed consent was collected prior to delivery. The verbal informed was authorized because not all women know to read, and those who know to read were not in good mood to read the consent, they were exposed to birth process.

Consent for publication

Not applicable

Availability of data and materials

All about the dataset are available via the corresponding author

Competing interests

There is no competent of interest declared

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Authors' contributions

CY: conceptualize the work, drafted the manuscript and analyzed data, JU: Data collection and records, CI: manuscript review, EM: assisted laboratory techniques, AOM: Conducted Data analysis, FN: a review

of the manuscript, JM: Laboratory techniques design, GBS: manuscript review, MN: Manuscript review, LM: worked on discussion, referencing and Conducted Data analysis. TH: reviewed the manuscript and Data analysis. All Authors have read and approved the manuscript.

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