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Study of chorioamnionitis among women with preterm birth at Ruhengeri referral hospital

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Abstract

Background: Chorioamnionitis is the association of microorganisms infection in fetal membrane, placental membrane and amniotic fluid. About 2 to 4% of chorioamnionitis occurs in full-term deliveries, but higher than this prevalence in preterm birth. **Objectives**: The objective of this study was to detect the most dominant bacteria of chorioamnionitis and evaluate correlation of the presence of microorganisms infection in placental membrane, fetal membrane and amniotic fluids in women with preterm birth. **Methods:** It was cross section study where 20 women with preterm premature rupture of membrane, premature rupture of membrane or preterm birth. By caesarean section or without caesarean section with a group of control of 10 women with term delivery. After delivery immediately the samples swab of amniotic fluid, fetal membrane and placenta membrane sample were placed in separate sterile container (swabs Stuart plastic) in the hospital, where it was stored at temperatures ranging between 8 ^{o}C-20 ^{o}C. Culture technique, gram staining and biochemical test were used to identify the microorganisms in this study at INES-Ruhengeri Microbiology laboratory. At the Ruhengeri Referral Hospital and analyzed according to INES Microbiology Laboratory standard operating procedures. **Results**: in 20 women with preterm birth, the most dominant mircoroganism was *yeast* 28.4% and *mould* 28.4%, other infections were caused by *Escherichia coli,* with 9.3%, *Klebsiella species* with 3.7%, *Streptococcus species* with 9.3%, *Staphylococcus species* with 9.3%, *Candida albican* with 11.7%. Those microorganisms show the association in fetal membrane, placenta membrane and amniotic fluid, of all women suspected to have chorioamnionitis. In 10 samples of control group, for women with term birth, same microorganisms were found like in fetal membrane were *(Staphylococcus species* 11%, *mould* 33%, and *Yeast* 56%), in placenta membrane were (*Staphylococcus species* 20%, *mould* 30%, *Yeast* 50%) and Amniotic fluid were *(Staphylococcus species* 0%, *mould* 33%, *Yeast* 67%). **Conclusion**: Chorioamnionitis can be in women with preterm birth or with term birth, untreated microorganism infections will cause a big problem of chorioamnionitis in pregnant women this will conduct to preterm morbidity and mortality.

Keywords: *Chrioamnionitis, Preterm birth, Microorganisms, Amniotic fluids, Placental membrane*

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1. Introduction

Chorioamnionitis is a term used to describe infections in the placental membranes and amniotic fluid (Rodriguez *et al*., 2016). The estimate shows that 2 to 4% of chorioamnionitis occurs in full-term deliveries, but more than this in preterm birth. Chorioamnionitis can be a leading cause of bacteremia (blood infection) in mothers and may lead to preterm birth and serious infection in neonates (Jane and Stuart, 2013). Other used terminologies used for chorioamnionitis include intra-amniotic infections and amnionitis. Chorioamnionitis or intra amniotic infection is also defined as an acute inflammation of the membranes and chorion of the placenta, typically due to the ascendance of polymicrobial bacterial infection in the setting of membrane rupture (Kim *et al*., 2007).

Ruptured membrane is not only a clinical feature of chorioamnionitis due to its occurrence in mothers with intact membranes, and in this case, the predominant microorganisms are very small, fastidious genital Mycoplasmas including Urea plasma species and Mycoplasma hominis, located in the lower genital tract, with the prevalence of over 70% of women (Doyle *et al*., 2017). The etiologic agents of chrioamnionionitis are microorganisms that mostly colonize and were found in the vaginal, including *Escherichia coli (E. coli)*. Group B streptococcus may also cause the infection (Czikk *et al*., 2011). Chorioamnionitis can develop when the membranes (amniotic sac) are ruptured (broken) for an extended period. This allows the vaginal organisms to move upward into the uterus (Cooke, 2008).

If the infection reaches the womb it can lead to serious problems on fetal membranes (membrane of the sac that surrounds a baby in the womb, also called amnion or chorion) like inflammation and other serious fetal outcomes like serious infections of the baby and this can result to death of the baby. This is what we call chorioamnionitis (Goldenberg *et al*., 2000). Overall the definition of chorioamnionitis varies according to key diagnostic criteria, which can be clinical (presence of typical clinical findings), microbiologic (culture of microbes from appropriately collected amniotic fluid or chorioamnionic) or histopathology (microscopic evidence of infection or inflammation on examination of the placenta or chorioamnionic specimens) (Henríquez and Rodrigo, 2017).

Clinical chorioamnionitis in pregnant women is diagnosed based on clinical outcomes (findings) like fever plus fetal tachycardia, maternal leukocytosis, or purulent fluid coming from the cervical os which is a part of the female reproductive system located in the pelvis (Horvath *et al*., 2014). In addition, the pregnant woman with chorioamnionitis can appear with illness, and there is occurrence of hypotension, diaphoresis, and/or cool or clammy skin.

However, clinical signs (features) and symptoms of chorioamnionitis are not always known to be associated with placental evidence of inflammation. Examination for suspected sepsis in the newborns of a mother suffered from chorioamnionitis results on nonspecific and subtle findings, including the following: Behavioral abnormalities (e.g., lethargy, hypotonia, weak cry, poor suck), Pulmonary: Tachypnea, respiratory distress, cyanosis, pulmonary hemorrhage, and/or apnea, Cardiovascular: Tachycardia, hypotension, prolonged capillary refill time, cool and clammy skin, pale or mottled appearance, and/or oliguria, Gastrointestinal: Abdominal distention, vomiting, diarrhea, and/or bloody stools, Central nervous system: Thermal regulatory abnormalities, behavioral abnormalities, apnea, and/or seizures, Hematologic and/or hepatic: Pallor, petechiae and overt bleeding (Apantaku and Mulik, 2007).

2. Materials and Methods

2.1. Study Area

Ruhengeri referral hospital began with the opening of a dispensary in 1964. Ruhengeri hospital upgraded from a district to a referral hospital in 2014. It began receiving cases from 15 health centers in its own district and from five hospitals in surrounding districts. In 2015 alone, the hospital experienced close to 6,000 monthly outpatients visits. Such numbers proved how important the status upgrade had been in relation to local health needs. Today, Ruhengeri referral hospital serves 250 patients per day, it is now approximately to 7,500 patients monthly. Ruhengeri referral hospital is located in Muhoza sector, Musanze district, Northern Province of Rwanda.

2.2. Study Design

This study was cross-sectional that aimed to identify common predominant microorganisms causing chorioamnionitis among mothers with preterm delivery based on the criteria of the preterm mothers delivering between 32 and 37 weeks' gestation because of Preterm Premature Rupture of Membranes (PPROM), spontaneous preterm labor, or clinically diagnosed chorioamnionitis, At Ruhengeri referral hospital.

2.3. Study Population

The research was conducted in mother with PPROM, PROM and PTB delivery attending Ruhengeri referral hospital in the period of study from November to December.

2.4. Sample Size

In this study the total population was 20 mothers with PPROM, PROM and PTB were suspected to have chorioamnionitis, 10 of mother with term delivery were considered as control.

2.5. Sample Collection

2.5.1. Sample Collection, Preparation and Storage

After delivery, immediately swab of amniotic fluid, fetal membrane and placenta sample were placed in separate sterile container (swabs Stuart plastic) in the hospital, where it was stored at temperatures ranging between $8^{\circ}C$-$20^{\circ}C$. After that on the next day containers were transported at INES-Ruhengeri microbiology laboratory where the collected samples were analyzed.

2.6. Microscopic Examination and Direct Examination

2.6.1. Wet Preparation

Through the use of fresh sample in Stuart swab was put on slide covered with cover slide and observed under microscopy many samples show the red and white cells, the yeasts, the motile and non-motile bacteria, if there is one of those microorganisms means that results was positive then we continue with gram stain and culture was used to identify the different microorganisms.

2.6.2. Gram Stain

Gram staining was used during this research to differentiate gram positive and gram negative. Slides are sequentially stained with crystal violet 30 seconds, rinse clear with tap water, and flood with Iodine solution in 30 seconds then rinse clear with tap water and then distained with alcohol in three seconds and counterstained with safranin in 30 seconds allow it to air dry. Gram positive bacteria stained blue-purple and Gram negative bacteria stained red.

2.6.3. Culturing the Samples

Blood agar, MacConkey, and Sabrod agar were used for different sample, this was done according to the group of bacteria have been found on gram stain for placental, fetal membrane and amniotic fluid. The media were prepared according to the manufacturer's instructions, and samples were inoculated into a plate containing medium using a loop from flame for disinfection. The plates were incubated at $37^{\circ}C$ for 24 h. After, the plates with growth colonies were observed and others returned to the incubator before discarding since other factors may inhibit initial growth for another 24 h.

2.7. Biochemical Test

2.7.1. Biochemical Test Used to Differentiate Gram Negative Bacteria

MacConkey agar which is differential and selective media was used for to isolate and differentiate Gram negative bacteria. In this test Simon's citrate agar was used to test an organism's ability to utilize citrate as a source of energy. Motility was confirmed when a wide filament-like form appeared in MIU broth medium. An appearance of a red ring-like form on its surface after adding two to three drops of Kovac's reagent indicated indole positive. Urease positive was proved by color change to pink. Kligler Iron Agar (KIA) permitted differentiation of Gram negative bacilli by their ability for fermenting glucose or lactose which produces color change of the PH indicator in response to acid production in the sugar fermentation.

2.7.2. Biochemical Test Used to Differentiate Gram Positive Bacteria

After 24 h of incubation at $37^{\circ}C$, bacterial growth on culture media was presented differently through colonial morphology characteristics. Based on the size, color, consistence, and shape, the type of bacteria on culture media was suspected. In this study, catalase and coagulase tests were used differentiate *Staphylococci* and *Streptococci* species gram positive bacteria.

2.8. Catalase

Catalase test was performed for differentiation of *Staphylococci* and *Streptococci spp.* that are all gram positive cocci bacteria. The mixture of Bacterial colonies and hydrogen peroxide (H_2O_2) was done and the enzyme

"catalase" was proved by raising the air bubbles. *Staphylococci* were catalase positive while *Streptococci* were catalase negative. *Streptococcus* were differentiated by its hemolysis where (α - hemolytic made up of two groups including *Streptococcus pneumonia,* β - hemolytic made up of many groups including group A and B, *Streptococcus agalactiae*).

2.9. Coagulase

Coagulase is an enzyme that clots blood plasma. This test was performed to differentiate *Staphylococcus species* by placing a drop of physiological saline on each end of a slide, with the loop, straight wire, emulsify a portion of the isolated colony in each drops to make two thick suspensions. A drop of human plasma was added to one of the suspension, and mix gently. Clumping of the organisms was checked within 10-15 seconds. The presence of agglutination indicated coagulase positive to prove *Staphylococcus aureus* while coagulase negative confirmed other *Staphylococcus species*.

2.10. Yeast and Mould

Sabrod agar is a selective media of yeast and mould, the media were prepared according to the manufacturer's instructions, and samples were inoculated into a plate containing medium using a loop from flame for disinfection. The plates were incubated at 37 °C for 24 h after, the plates with growth colonies were observed under microscopy. If yeasts are present we continue with gem-tube test which were incubated for 8 h, after we prepare the wet amount for yeast differentiation (Candida albicans and other species) and mould.

2.11. Data Analysis

After conducting the study on association of chorioamnionitis and preterm birth the data was analyzed by using Microsoft Office Excel 2010 and was presented in the tables and figures.

2.12. Ethical Consideration

An authorization letter from Ines-Ruhengeri was given to General Director of Ruhengeri referral hospital. After getting permission, data collection was granted and the study begun. The study number was used instead of patient's names for the confidentiality of the participants and they were informed about their result, and also a copy of result was given at Ruhengeri referral hospital after the study.

3. Results

This chapter will be focused on data collected at Ruhengeri referral hospital and analyzed at INES microbiology laboratory. Results interpretation was presented in tables and figures.

Table 1: Demographic characteristics of women suspected to have chorioamnionitis

Age Group	Frequency	Percentage
19-23	5	17
24-29	15	50
30-34	4	13
35-39	6	20
Total	**30**	**100**

3.1. Demographic Characteristics of Women Suspected to have Chorioamnionitis

Table 1 shows the demographic characteristic of women suspected to have chorioamnionitis.

Result from this Table 1 show the demographic characteristic in women suspected to have chorioamnionitis and women with term birth between 19-23 were 17%, 24-29 were 50%, 30-34 were 13%, and 35-39 were 20%.

3.2. Identified Microorganisms in Women Suspected to have Chorioamnionitis

This figure represents microorganisms isolated from women suspected to have chorioamnionitis.

Results from Figure 1 indicates that *Escherichia coli* was 15 (9.3%), *Klebsiella species* was 6 (3.7%), *Streptococcus species* was 15 (9.3%), *Staphylococcus species* was 15 (9.3%), *mould* was 46 (28.4%), *Yeast* was 46 (28.4%), *Candida albican* was 19 (11.7%).

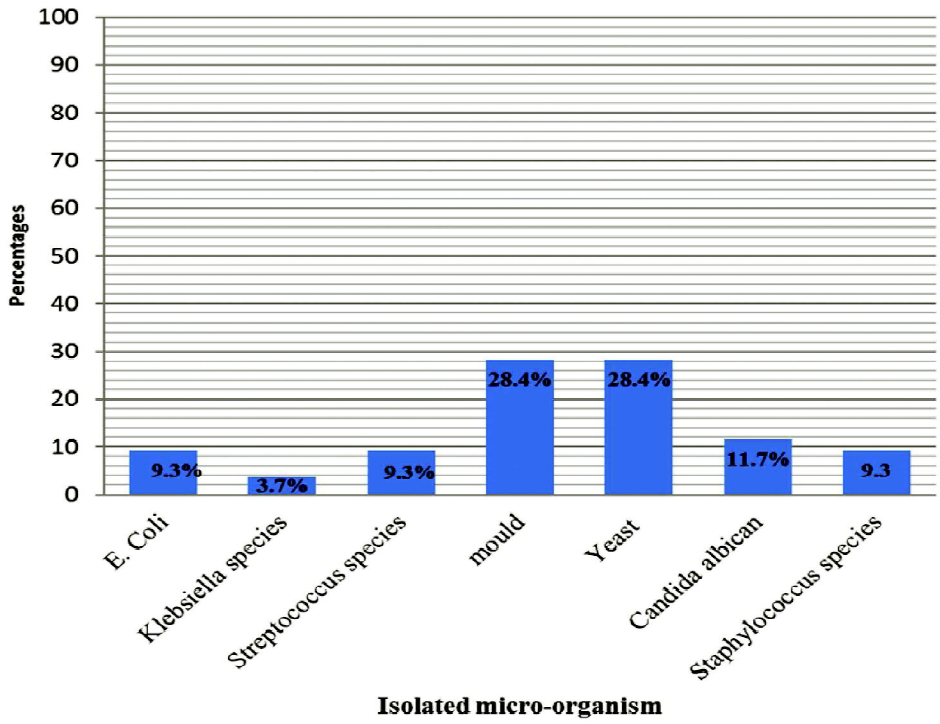

Figure 1: Identified microorganisms in women suspected to have chorioamnionitis

3.3. The Most Dominant Microorganisms Infection Among Preterm Birth Mothers

Figure 2 shows dominant microorganisms identified in women with chorioamnionitis in placenta membrane the following microorganisms have been identified (*Escherichia coli* 8.6%, *Klebsiella species* 5.2%, *Streptococcus*

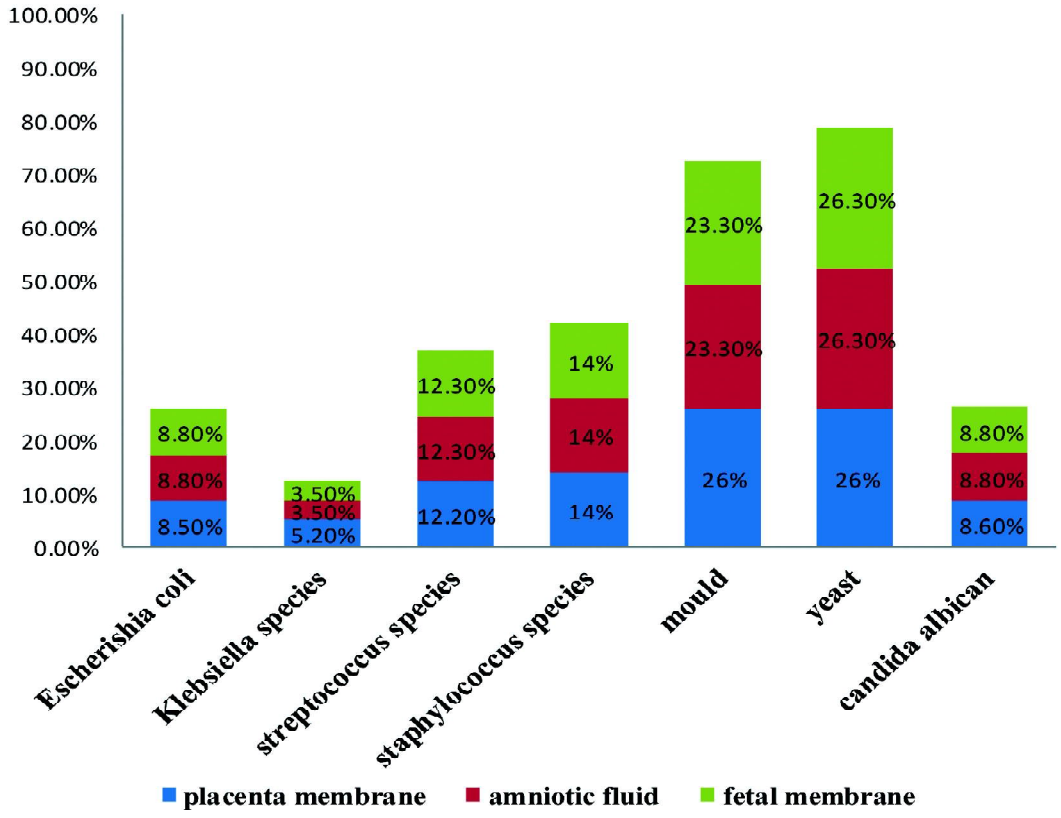

Figure 2: The most dominant microorganisms infection in women with PTB

species 12.1%, *Staphylococcus species* 14.0%, *mould* 23.3%, *Yeast* 26.3% and *Candida albican* 8.6%), in amniotic fluid was *(Escherichia coli* 8.8%, *Klebsiella species* 3.5%, *Streptococcus species* 12.3%, *Staphylococcus species* 14.0%, *mould* 23.3%, *Yeast* 26.3% and *Candida albican* 8.8%), and in fetal membrane was *(Escherichia coli* 8.8%,*Klebsiella species* 3.5%, *Streptococcus species* 12.3%, *Staphylococcus species* 14.0%, *mould* 23.3%, *Yeast* 26.3% and *Candida albican* 8.8%).

3.4. The Most Dominant Microorganisms Isolated from Women with Term Birth

The result in Figure 3 above show microorganisms in fetal membrane were *(Staphylococcus species* 11%, *mould* 33%, and *Yeast* 56%), in placenta membrane were (*Staphylococcus species* 20%, *mould* 30%, *Yeast* 50%) and Amniotic fluid were *(Staphylococcus species* 0%, *mould* 33%, *Yeast* 67%). Microorganisms can be found in fetal not in amniotic fluid by contamination especially in this case of women with term delivery. According to the stage of infection, in this case a baby can be contaminated in vaginal at delivery time.

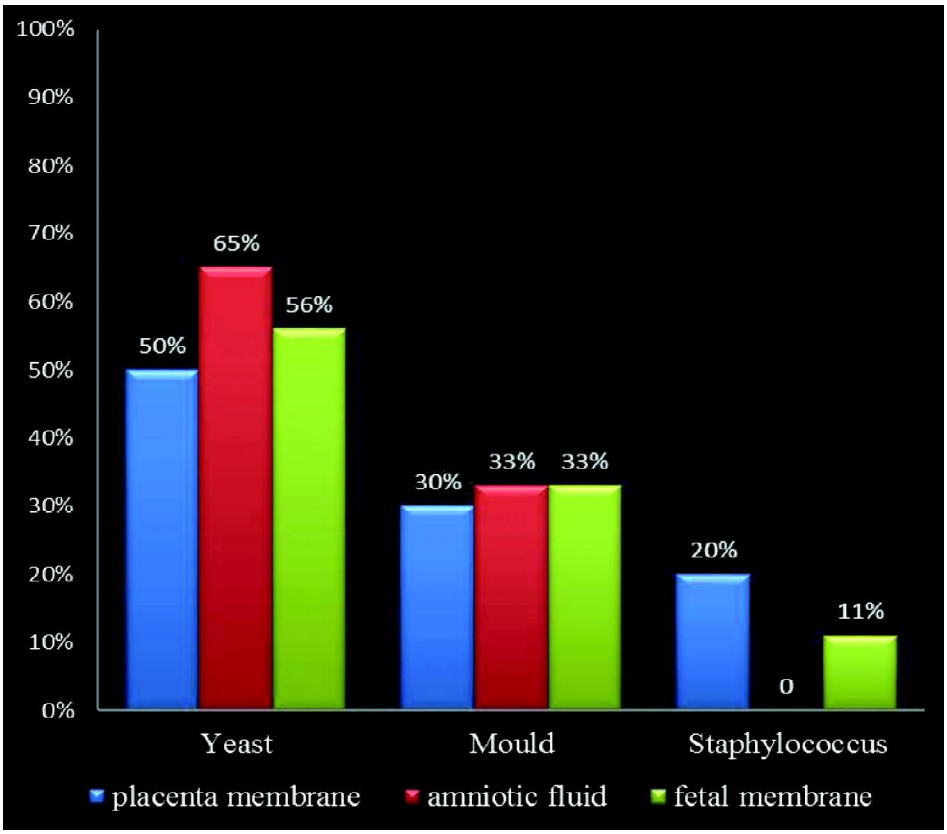

Figure 3: The most dominant microorganisms isolated from women with term birth

4. Discussion

4.1. The Most Dominant Isolated Microorganisms in Women With Preterm Birth and Term Birth

In this study conducted at Ruhengeri referral hospital in 2018, the result of 20 mothers with PPROM, PROM and PTB, show that approximately, 28.4% of *mould* and *Yeast* was the dominant microorganisms. Followed 11.7% of *Candida albican*, 9.3% of *Streptococcus* species and *Staphylococcus* species 9.3% of *Escherichia coli* and 3.7of *Klebsiella* species. In 10 samples *Staphylococcus* species, *mould* and *Yeast* were microorganisms isolated in control group which means that chorioamnionitis can be in women with term delivery.

This finding had similarity from the data reported by the study conducted by Kikhney *et al.* (2017). In Germany For 20 women in the preterm group, caesarean section was performed because of a clinical diagnosis of chorioamnionitis. Microorganisms were detected in the tissues by both molecular techniques in 11 out of 20 women. Among those, Ureaplasma spp. was most abundant, with five cases that remained culture negative and would have been missed by routine diagnostic procedures (Kikhney *et al.*, 2017).

Other infections were caused by *Staphylococcus aureus*, *Streptococcus mitis* or *Escherichia coli*. FISH and PCR were negative for all women without suspected chorioamnionitis and for the control group (Cappelletti *et al.*,

2016). Fungal organisms, including several species of Candida (*Candida albicans*, Candida tropicalis and Candida glabrata), have also been associated with chorioamnionitis. However, the complications of intraamniotic fungal infection can be severe, with a 75% risk of prematurity being associated with candida. The issue for preterm delivery mothers were that the frequency of microorganisms were high compared to term delivery mothers which show strong association between chorioamnionitis and PTB.

4.2. Comparisons of Microorganisms Infection in Placental Membrane, Fetal Membrane and Amniotic Fluids

Correlation of microorganisms infection in this research done at Ruhengeri referral hospital and at Ines microbiology laboratory in 2018 the result in fetal membrane was *(Escherichia coli* 8.8%, *Klebsiella species* 3.5%, *Streptococcus species* 12.3%, *Staphylococcus species* 14.0%, *mould* 23.3%, *Yeast* 26.3% and *Candida albican* 8.8%), in placenta membrane was (*Escherichia coli* 8.6%, *Klebsiella species* 5.2%, *Streptococcus species* 12.1%,*Staphylococcus species* 14.0%, *mould* 23.3%,*Yeast* 26.3% and *Candida albican* 8.6%) and Amniotic fluid was *(Escherichia coli* 8.8%, *Klebsiella species* 3.5%, *Streptococcus species* 12.3%, *Staphylococcus species* 14.0%, *mould* 23.3%,*Yeast* 26.3% and *Candida albican* 8.8%). It was seen that number of microorganisms found in samples were equal or approximately equal, and it shows easy migration of infection from placental to fetal membrane. When we compare this study with the studies conducted by Doyle (2016) in Malawi used microbial culture techniques to sample and diagnose the presence of bacteria in the amniotic fluid and choriodecidual space.

Many preterm deliveries showed signs of inflammation in a number of the maternal and fetal tissues but there was no evidence of infection by culture. This made diagnosing intrauterine infection as a potential cause of preterm increasingly difficult. During this period, researchers found that measuring cytokine levels such as interleukin-6 was a better predictor of preterm birth than the presence or absence of an infectious agent.

Either culture techniques were not sensitive enough to elucidate possible infection or microbial invasion of the amniotic cavity is not the only infection site that triggers preterm birth. The limitations of classic bacteriology are well-documented and many microbial species are uncultivable or difficult to culture at this time. Analysis of intrauterine infection by this technique is thought to be underestimating the problem. However, when bacteria have been cultured it was found that intra-amniotic infection was often poly microbial with multiple genera recovered including *Ureaplasma*, *Mycoplasma*, *Fusobacterium*, *Bacteroides*, *Streptococcus* and *Gardnerella*.

5. Conclusion and Recommendations

5.1. Conclusion

This study aimed to identified chorioamnionitis dominant microoganism in preterm birth demographically to evaluate the correlation of the presence of microorganisms infection in placental membrane, fetal membrane and amniotic fluids in mothers who give birth at Ruhengeri referral hospital after conducting the study the mostly microoganism observed in placental membrane, fetal membrane and amniotic fluids were *E. coli*, *Klebseilla*, for Gram negative bacteria, *staphylococcus* ssp, *streptococcus ssp* for Gram positive bacteria, *mould*, *yeast* ssp. and *Candida albican* for fungi and chorioamnionitis is caused by those microorganisms, which means that chorioamnionitis is one of the major cause of preterm birth at Ruhengeri referral hospital.

5.2. Recommendations

5.2.1. To the Ministry of Health

To add more efforts and capabilities to the nurses, midwives and others that may be concerned from health centers to the hospitals through seminars. This will enable them to make a clear follow-up to the pregnant mothers showing them how they can prevent and fight against bacterial infections that may result in chorioamnionitis and can affect their siblings.

5.2.2. To Ruhengeri Referral Hospital

To request exams for diagnosis of bacterial infections in pregnant mothers from the first day of pregnancy to the day of delivery. By doing culture and antibiogram to prevent same resistance of microorganisms to same antibiotic that may cure those infection which may cause chorioamnionits in pregnant women.

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