RESEARCH
O&G Forum 2024; 34 – 3s: 945-952

EXPLORATION OF THE VAGINAL MICROBIOME COMPOSITION IN PARTURIENT WOMEN ADMITTED TO A TERTIARY CARE HOSPITAL: A COMPREHENSIVE INVESTIGATION

Heikham Gineta Chanu [1*], Dr. Sangeeta N. Kharde [2], Dr. Anita Dalal [3], Dr. Suneel Dodamani [4], Shivani Tendulkar [5], Mehul A. Shah [6]
[1] Assistant Professor, KAHER Institute of Nursing Sciences, Belagavi, India. genitachanu@gmail.com
[2] Professor & Head, Department of OBG Nursing, KAHER Institute of Nursing Sciences, Belagavi, India. sangeeta.kharde@gmail.com
[3] Dr. Anita Dalal, Professor & Head, Obst & Gynecology, JNMC, Belagavi, India. anitamgan@gmail.com
[4] Scientist- I, Dr. Prabhakar Kore's Basic Science Research Center College, KLE Academy of Higher Education and Research, Belagavi, India. suneelddmn18@gmail.com
[5] Ph.D Scholar, BSRC, KAHER, Belagavi, India. tendulkarshivani@gmail.com
[6] Assistant Professor, Department of Public Health Dentistry, Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, Pune, Maharashtra, India. mehulshah1126@gmail.com

Abstract
Background: Vaginal microbiome is the microorganism that colonizes the vagina and has a significant impact on women's health and disease conditions. Microbiome starts to develop from the uterus and is further influenced by various factors like timing of birth, environmental exposures, diet, inflammatory immune responses, clinical infection, and sexual and hygiene practices. Anaerobic bacteria replace the lactobacillus, the usual vaginal flora, in bacterial vaginosis. About 20% of expectant mothers have it, and most of them have no symptoms. It is associated with premature rupture of membranes, preterm delivery, chorioamnionitis, and intra-uterine death. It also leads to adverse neonatal outcomes like assisted ventilation or respiratory distress at birth, neonatal Intensive care unit admission, fetal and infant mortality, neonatal sepsis, chronic lung disease and delayed development.
Methods: A case series study was conducted among 300 women to study the vaginal microbiome in women admitted for delivery to a tertiary care hospital at Belagavi. Vaginal swabs were collected from the posterior vaginal fornix and then placed in a sterile tube which was stored immediately until the sample is tested at -70°C. The samples were smeared onto MRS agar plates and left in an anaerobic room for a whole day at 370C. A biochemical test (Gram staining and IMViC test) was performed for the samples followed by DNA extraction using QIAamp ® DNA Mini Kit. After the amplification, the samples were analyzed by Sanger Sequencing.
Results: Gram staining was performed for all the samples followed by IMVIC test. Out of all samples, 106 were Voges-Proskauer test positive and 69 were citrate test positive. In the study, 18 samples (6%) were gram negative and 282 samples (94%) were gram positive. After identifying the samples with similar characteristics, representatives were selected for DNA isolation and then sanger sequencing was performed. The result showed that the women who had full term delivery showed more abundance of L. crispatus (39.3%), L. jensenii (27.8%), L.iners (26.3%), Bacillus cereus (6.6%); and the women who had preterm delivery showed the presence of Lactobacillus iners (48.8%), L.Gasseri (7.3%), Atopobium vaginae (4.9%) and Enterococcus faecalis (39%).
Conclusion: Women who may benefit from interventions targeted at restoring a normal vaginal flora and who are at risk of premature delivery can be identified with the aid of early detection of aerobic vaginitis and bacterial vaginosis in pregnancy. A large population study will be needed to ascertain whether treating vaginal dysbiosis in asymptomatic pregnant women will have a meaningful impact on the onset of premature labour. The study suggests imparting awareness to health care professionals and all women of reproductive age group regarding the importance of maintaining healthy vaginal microbiome
Keyword: Child birth, Full-term labour, low birth weight, preterm labour, vaginal microbiome.

INTRODUCTION

Reproductive health of a women is important not only for her health but also for her partner/husband and child. Bacterial

infections may affect pregnant women from the time of implantation of fertilized ovum till delivery and also during the postpartum period.[1]

The vaginal microbiome or vaginal flora is the micro-organisms that colonize the vagina. It is thought to have a significant impact on women's health and illness states. A woman in the reproductive age range secretes between 1 and 4 milliliters of vaginal fluid, each of which has 106–108 bacteria per milliliter. The vaginal microbiome is dynamic due to the composition that changes during pregnancy, menstruation and diseases like bacterial vaginosis.[2,3]

The development of the microbiome begins in utero and is further impacted by several factors, including the time of birth (i.e., immediately after or at the gestational age), the mode of delivery, the use of antibiotics, the use of breast milk,[4] exposure to the environment, nutrition and diet, inflammatory immune responses, clinical infections, disease state[5], and sexual and hygiene practices.[6] During pregnancy, *Lactobacillus lactis* lowers the presence of common bacterial species due to the intravaginal cleansing effect. In some women, there may be some microorganisms other than the normal microorganisms that can cause complications.[7,8]

Anaerobic bacteria such as *Gardnerella vaginalis* and *Mycoplasma hominis* replace the typical *Lactobacillus vaginalis* flora in cases of bacterial vaginosis. About 20% of pregnant women have it, and most of them have no symptoms. It is associated with premature rupture of membranes, preterm delivery, chorioamnionitis and intra-uterine death. Studies have shown that poor pregnancy outcomes are higher among pregnant women with bacterial vaginosis and urinary tract infections. Bacterial vaginosis can lead to adverse neonatal outcomes like assisted ventilation or respiratory distress at birth, neonatal Intensive care unit admission, fetal and infant mortality, neonatal sepsis, chronic lung disease and delayed development.[1, 9]

Vaginal microbiome varies among the asymptomatic women belonging to reproductive age group.[10, 11] The frequency of bacterial vaginosis ranges from 4% to 64% worldwide. In women who are of reproductive age, it is the most prevalent lower vaginal tract infection.[12]

Although there is no conclusive data, studies have indicated that treating bacterial vaginosis during pregnancy to lessen the related problems is not very beneficial.[13, 14]

Many studies have been done on the vaginal microbiome (microorganisms) and their links to preterm birth, but considerably less research has been done on the changes that occur in the vaginal microbiome during late pregnancy and childbirth. Despite much investigation, the underlying causes of the triggering of labour remain unknown. Thus, an interesting area of study is the potential correlations between the vaginal microbiome in late pregnancy, shortly before childbirth, and at the commencement of labour. The mother's vaginal microbiome appears to alter during childbirth and following the rupture of the membranes, and the technique of delivery is known to impact the composition of the baby's future microbiome. But the scope and associated relationships are still mostly unclear.[15]

Hence this study is planned to find out the effect of vaginal microbiome on gestational age and outcomes of vaginal microbiome on mother and the baby. This will also help to make a protocol and educate the women on prevention of vaginal infection so that further complications will be prevented to the mother and fetus. This study will also help mothers to understand the importance of maternal reproductive health for positive pregnancy outcomes.

MATERIALS AND METHODS

The study's research methodology was an evaluative method. Utilising a case series research design, consecutive mothers admitted for delivery in selected tertiary care hospital of Belagavi city were incorporated into the research. The formula used to determine the sample size was:

$$N=Z_{1-\alpha/2}\ pq \quad X\ 1.1$$
$$(10\%p)^2$$

Where,

$Z_{1-\alpha/2}$= one tail standard normal variate assuming sample size at 95% confidence interval;

P= Prevalence;

Q=1-p and Attrition= 15%

Considering the above formula, the sample size is calculated as 300.

The study included mothers with or more than 28 gestational weeks delivering in tertiary care hospitals of Belagavi city. The study excluded mothers using vaginal antimicrobials or antibiotics and those associated with medical conditions (hypertensive disorders in pregnancy, diabetes, heart disease, antepartum hemorrhage).

Ethical clearance and formal permission were obtained from the Institutional ethical committee and also from Medical Director and Medical Superintendent of the selected tertiary care hospital. Written consent from each participant was acquired. Mothers and family members were informed of the study's goal during the data collection process. The tools used were: Socio-demographic profile, Identification of bacteria by: Collection of sample (vaginal swabs), morphology testing, bio-chemical test, isolation of DNA, PCR (Polymerase chain Reaction) test.

After collecting vaginal swabs from the posterior vaginal fornix, they were promptly preserved at -70°C in a sterile tube until analysis. The samples were smeared onto MRS agar plates and left in an anaerobic room for 24 hours at 37^0C. These specimens underwent a biochemical examination (Gram staining and IMViC test).

Using the QIAamp ® DNA Mini Kit, DNA extraction was carried out by following the instructions below: Samples were put into a 1.5 millilitre microcentrifuge container. Following a thorough vortex to mix in 20 µl of Proteinase K and 180 µl of Buffer ATL, the mixture was incubated at 56 °C for three hours, or until fully lysed. After adding 200 µl of Buffer AL, mix well by vortexing for 15 seconds. Next, it was incubated for ten minutes at 70°C. A short centrifugation of the tube was used to remove the drips from the lid. Add 200 µl of 96–100% ethanol, and vortexed for 15 seconds. Centrifuging the tube for a short while extracted the drops from the cap. After being pipetted onto the QIAamp Mini spin column (in a 2 ml collection tube), the liquid was spun for one minute at 6000 x g (8000 rpm). After that, the flow-through and collection tube was thrown away. 500 µl of Buffer AW1 was introduced to a fresh 2 ml collection tube containing the QIAamp Mini spin column. The tube was centrifuged at 6000 x g (8000 rpm) for a duration of one minute. Once more, the collecting and flow-through tube was thrown away. 500 µl of Buffer AW2 was once more added to a fresh 2 ml collection tube containing the QIAamp Mini spin column. After that, the tube was centrifuged for three minutes at maximum speed (20,000 x g; 14,000 rpm). The tube used for collection and flow-through was disposed of. A fresh 1.5 ml microcentrifuge tube was filled with the QIAamp Mini spin column and distilled water. To elute the DNA, the tube was centrifuged at 6000 x g (8000 rpm) for 1 minute after being incubated for 1 minute at room temperature. A single band of

high-molecular weight DNA was seen on a 1.0% agarose gel used to assess the purity of the isolated DNA.

Table no.1: Primers used for PCR Amplification

S.No	Primer Name	Sequence 5' to 3'	Length
1)	16S rRNA- F	F (5'-AGAGTTTGATCCTGGCTCAG-3')	20
2)	16S rRNA- R	R (5'-GGTTACCTTGTTACGACTT-3')	19

The primers used for PCR Amplification are mentioned in (Table 1). 16SrRNA-F and 16SrRNA-R primers were used to amplify a fragment of the 16S rRNA gene. A single distinct 1500 bp PCR amplicon band was seen on an agarose gel. The PCR amplicon was purified to remove contaminants. Utilising the BDT v3.1 Cycle sequencing kit on an ABI 3730xl Genetic Analyzer, the forward and reverse DNA sequencing reactions of the PCR amplicon were performed using 16SrRNA-F and 16SrRNA-R primers. Using aligner software, a consensus sequence for the 16S rRNA gene was produced from forward and reverse sequence data. BLAST was performed using the 16S rRNA gene sequence and the NCBI GenBank database's "nr" database. Based on the maximum identity score, the first ten sequences were selected and aligned using the multiple alignment software Clustal W. Using MEGA 10, a distance matrix and phylogenetic tree were created.

The sequence obtained from the sequencing analysis was subjected to BLAST on NCBI. A nucleotide blast was conducted. The accession portion contained the forward and reverse nucleotide sequences. The organism was added in the allotted section and later clicked on blast. Similarly, in the same section, we also found the distance between the trees.

Operational definitions

- **Vaginal microbiome:** In the present study vaginal microbiome are the microorganisms present in the vagina that may or may not affect the gestational age or health status or of the women and also the health of the fetus and newborn.
- **Bacterial vaginosis:** In the present study, bacterial vaginosis means any imbalance of normal vaginal flora or microorganisms.
- **Preterm birth:** In the present study, preterm birth means delivery of babies before 37 full weeks of gestation.
- **Vaginal smear:** In the present study, vaginal smear means the vaginal sample collected in the cotton swab.
- **PCR (Polymerase chain reaction) Test:** It is a test to find genetic material from a particular organism, such as bacteria or a virus.
- **Lactobacilli:** In the present study, lactobacilli mean the normal vaginal flora.

RESULTS

Gram staining was performed for all the samples followed by IMVIC test. The analysis results of the biochemical test are mentioned in Table no. 2 and Table no.3.

Table no. 2: Biochemical Test (Gram Staining and IMViC Test) result for selected samples

Sample	Gram Staining			IMViC Test			
	Colour	Shape	Gram Reaction	Indole test	Methyl red test	Voges-Proskauer test	Citrate utilization test
1.	Violet	Rod	Gram +	-	-	-	-
2.	Blue	Rod	Gram +	-	-	-	-
3.	Blue	Rod	Gram +	-	-	-	-
4.	Purple	Rod	Gram +	-	-	-	-
5.	Blue	Rod	Gram +	-	-	-	-
6.	Purple	Rod	Gram +	-	-	+	+
7.	Purple	Rod	Gram +	-	-	+	+
8.	Blue	Oval	Gram +	-	-	+	-
9.	Blue	Oval	Gram +	-	-	+	-
10.	Blue	Oval	Gram +	-	-	+	-
11.	Blue	Rod	Gram +	-	-	-	-
12.	Blue	Rod	Gram +	-	-	-	-
13.	Blue	Rod	Gram +	-	-	-	-

14.	Blue	Rod	Gram +	-	-	-	-
15.	Violet	Rod	Gram +	-	-	-	-

Table no. 3: Biochemical Test (Gram Staining) Result for all samples

Bacteria	n	%
Gram Negative	**18**	**6**
Gram Positive	**282**	**94**
Total	**300**	**100**

Out of all samples, **106** were Voges-Proskauer test positive and **69** were citrate test positive.

16S rRNA Amplification and Sequencing:

After identifying the samples with similar characteristics, representatives were selected for DNA isolation and sent for sanger sequencing. The 16SrRNA amplicon of selected samples are shown in Figure no.1.

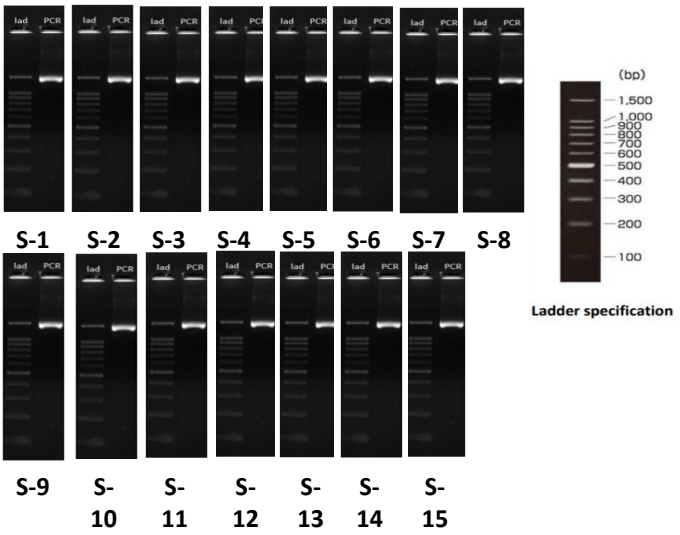


Fig no.1: 16SrRNA amplicon of selected samples

Table no. 4: Showing results of PCR amplification

Preterm	n	%	Term	n	%
L. iners	20	48.8	L.iners	68	26.3
L.gasseri	3	7.3	L. crispatus	102	39.3
Atopobium vaginae	2	4.9	L. jensenii	72	27.8
E. fecalis	16	39	Bacillus cereus	17	6.6
Total	41	100	Total	259	100

After the amplification, the samples were analyzed by Sanger Sequencing. The samples showed the presence of *Lactobacillus Crispatus, Lactobacillus jensenii, Lactobacillus inners, L. Gasseri, Bacillus cereus, Bacillus sp. Firmi, Bacillus sp. Strain, Atopobium vaginae, Enterococcus sp. Strain and Enterococcus faecalis.*

In the study, as mentioned in Table No. 4, the women who had full term delivery showed more abundance of *L. crispatus, L. jensenii, L. iners, Bacillus cereus , Bacillus sp. Firmi* and *Bacillus sp.* Strain; and the women who had preterm delivery showed the presence of *Lactobacillus iners, L.Gasseri, Atopobium vaginae, Enterococcus sp. Strain* and *Enterococcus faecalis.*

Phylogenetic tree: Phylogenetic tree of selected samples are shown in Figure no.2 to Figure no.16.

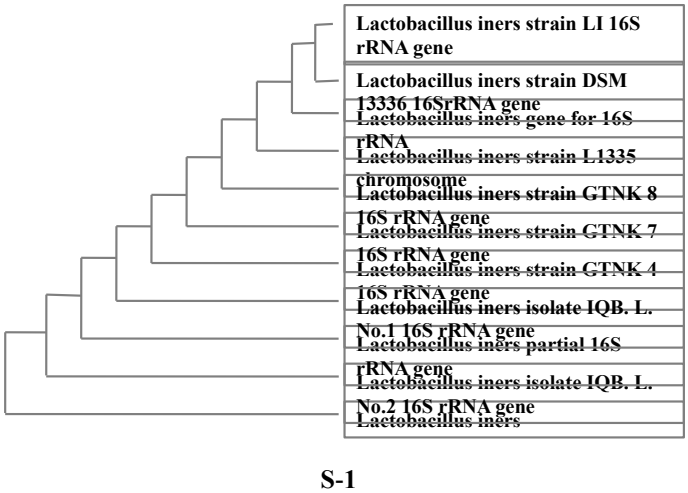


Fig no.2: Phylogenetic tree of Sample no.1

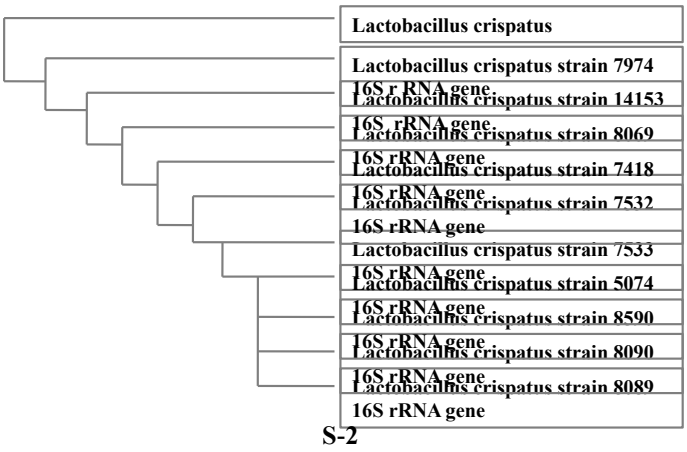


Fig no.3: Phylogenetic tree of Sample no.2

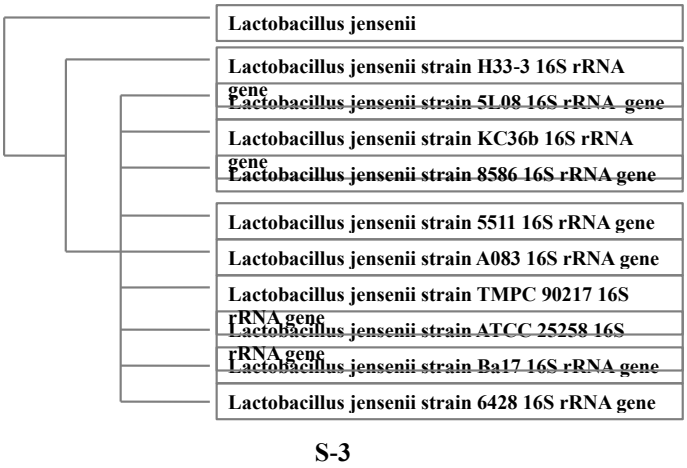


Fig no.4: Phylogenetic tree of Sample no.3

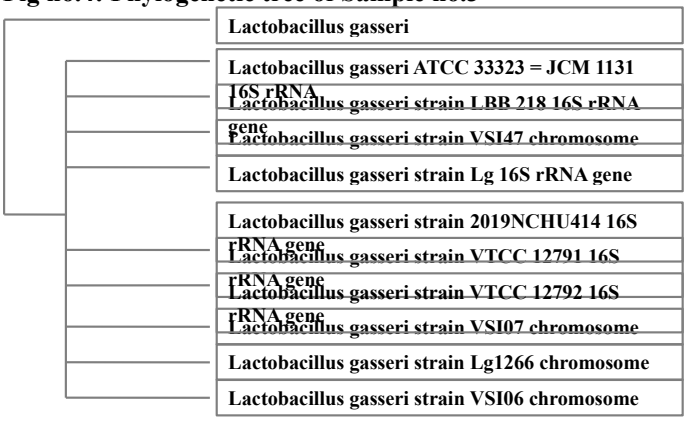


Fig no.5: Phylogenetic tree of Sample no.4

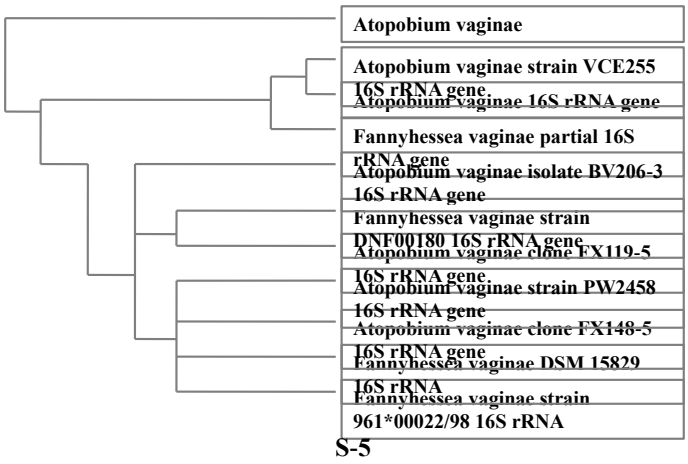


Fig no.6: Phylogenetic tree of Sample no.5

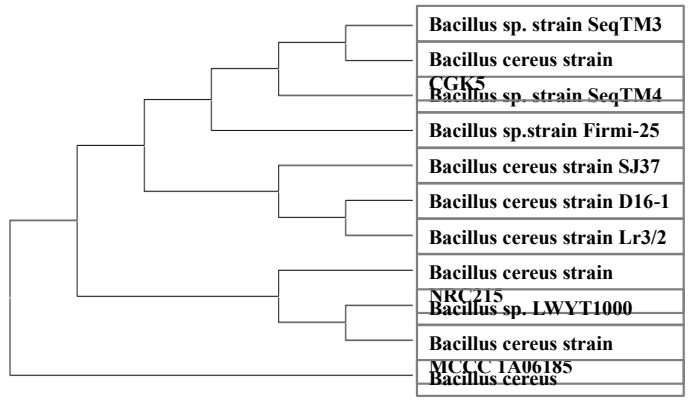


Fig no.7: Phylogenetic tree of Sample no.6

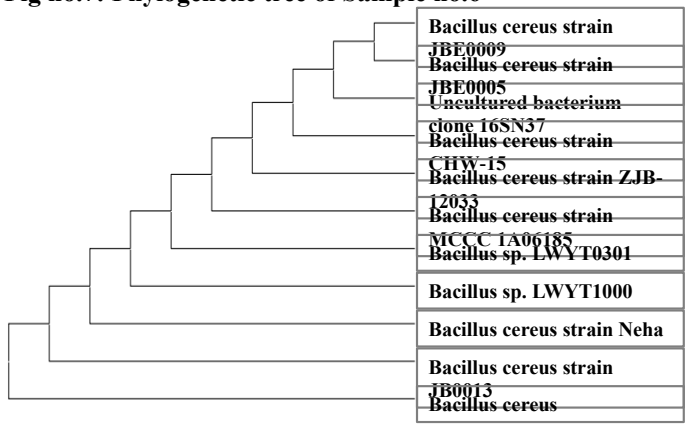
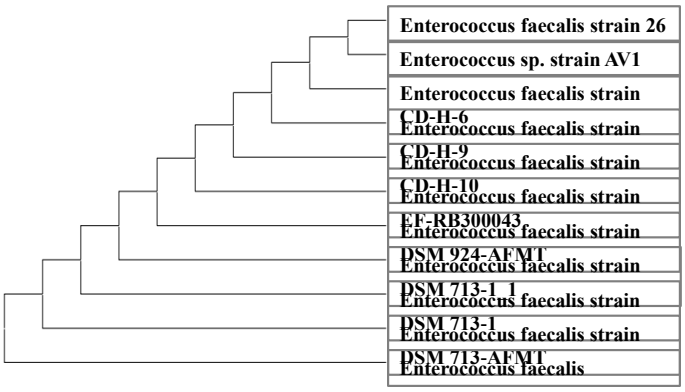
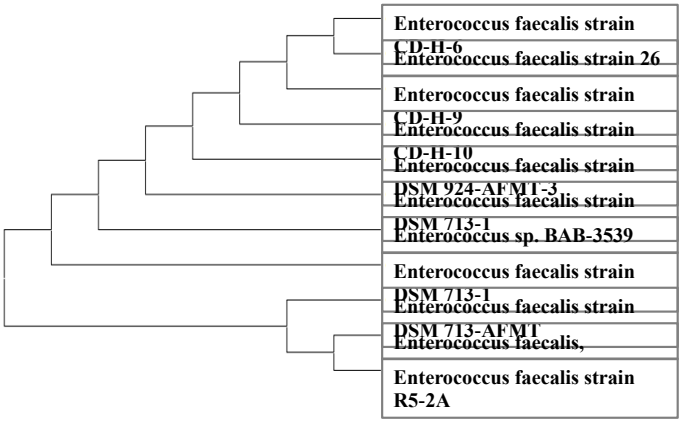


Fig no.8: Phylogenetic tree of Sample no.7



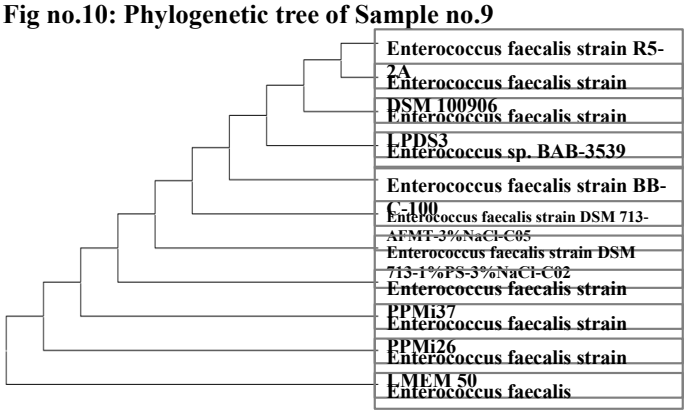
S-8

Fig no.9: Phylogenetic tree of Sample no.8



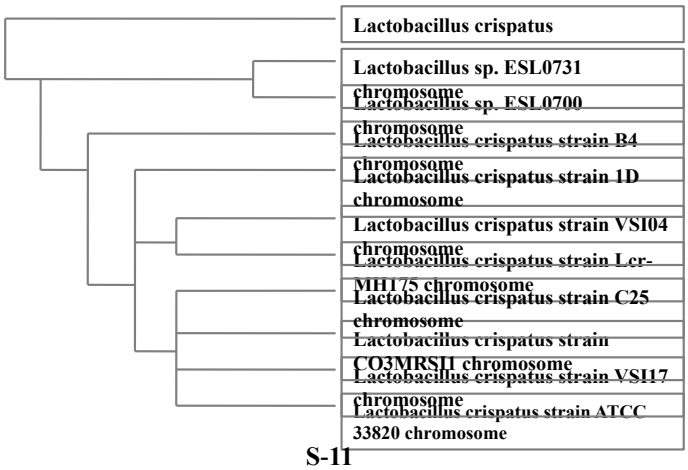
S-9

Fig no.10: Phylogenetic tree of Sample no.9



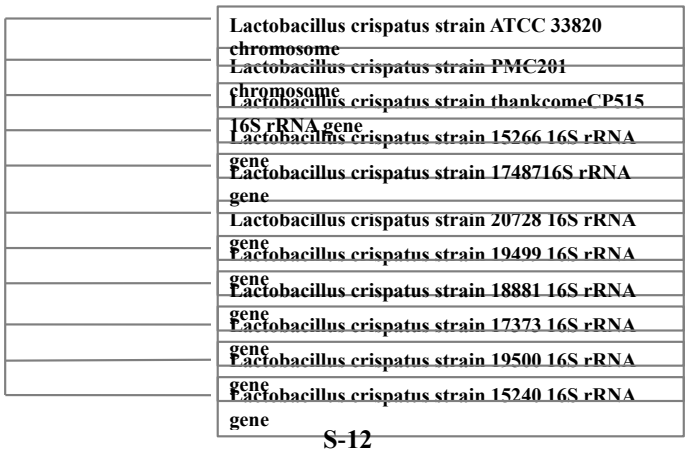
S-10

Fig no.11: Phylogenetic tree of Sample no.10



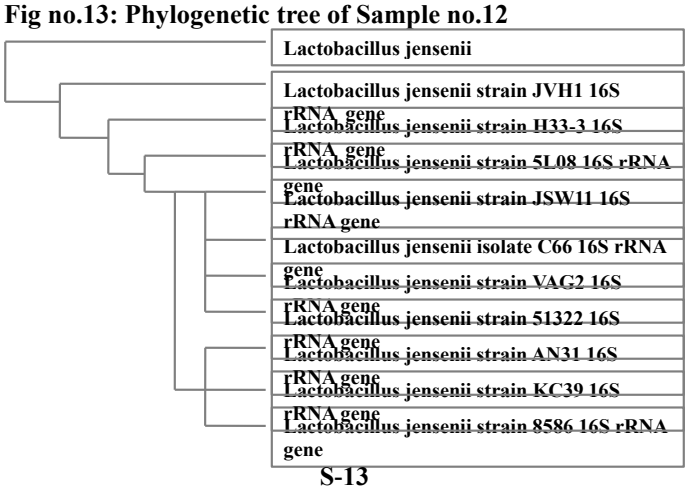
S-11

Fig no.12: Phylogenetic tree of Sample no.11



S-12

Fig no.13: Phylogenetic tree of Sample no.12



S-13

Fig no.14: Phylogenetic tree of Sample no.13

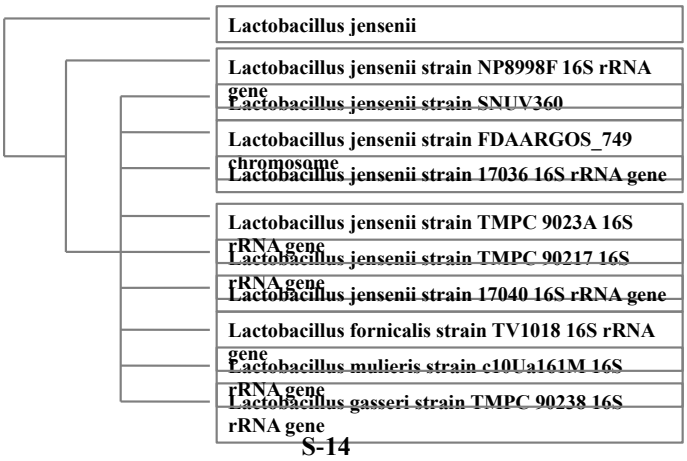


Fig no.15: Phylogenetic tree of Sample no.14

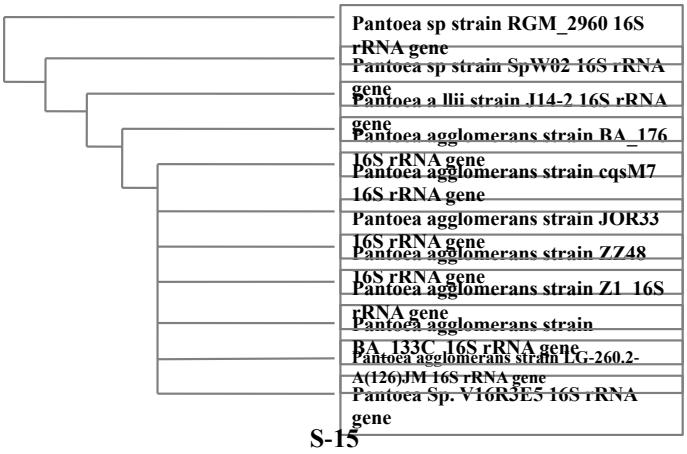


Fig no.16: Phylogenetic tree of Sample no.15

DISCUSSION

Lactobacillus species predominate in a healthy or ecologically stable vaginal microbiome, which regulates the pH of the vagina to help avoid genital infections. On the other hand, an imbalance in the microbial makeup of the vagina is known as an aberrant vaginal microbial composition, and it is linked to a higher risk of trichomoniasis, bacterial vaginosis, STDs, premature labour, and other birth abnormalities. Race, ethnicity, pregnancy, hormonal fluctuations, sexual activity, cleanliness habits, and other factors all have an impact on this microbial diversity.[16] It's also possible that variations in the prevalence of different Lactobacillus strains in the vaginal microbiome are just the result of ethnic variances.[17]

The predominant *Lactobacillus Acidophilus, Lactobacillus Crispatus, Lactobacillus Gasseri, Lactobacillus Jensenii,* and *Lactobacillus Iners* species found in the vaginal flora. A change in the vaginal flora can cause anaerobic species such *Gardnerella, Mobiluncus, Bacteroides, Prevotella, and Peptostreptococcus* to predominate, along with *Streptococcus, Staphlylococcus*, and *Escherichia coli.* This can lead to either an aerobic vaginitis or a bacterial vaginosis. It is common for fertile women to contract bacterial vaginosis. A vaginal discharge that has the characteristic "fishy" smell is a sign that it is present. This is brought on by the amines that bacteria create during metabolism.[18]

In this study, the women who had full-term delivery showed more abundance of *L. crispatus, L. jensenii, L. iners and Bacillus cereus*; and the women who had preterm delivery showed the presence of *L. iners, L.Gasseri, Atopobium vaginae and Enterococcus faecalis*. Sakabe Y et al. conducted a similar investigation and discovered that Lactobacillus was generally plentiful, with *L. crispatus and L. iners* being particularly common, whereas the population of *L. gasseri* was low in samples of preterm deliveries.[19] It was discovered that *L. crispatus* dominated the vaginal microbiota of healthy women before or during pregnancy. However, during the puerperium, the situation changed, resulting in a drop in the number of disease-fighting Lactobacillus species, making the vaginal microecological barrier vulnerable to illnesses.[20] In another study, it was discovered that term-delivering mothers have one or more Lactobacillus species like *L. crispatus* followed by *L. jensenii* and *L. gasseri* whereas 40% of preterm-delivering mothers lack *Lactobacillus species*.[21]

Randomization could not be done in the present study which stands out as the limitation of our study. The following future recommendations can be considered: To enable a wider generalization of the findings, a comparable study with a bigger sample size might be conducted. A similar study can be conducted in different settings and also by using a randomization sampling technique.

CONCLUSION

Each woman admitted to the hospital for early labor had a diagnosis of the altered normal vaginal microbiota. Women who may benefit from interventions targeted at restoring normal vaginal flora and who are at risk of premature delivery can be identified with the aid of early detection of aerobic vaginitis and bacterial vaginosis in pregnancy. A large population study will be needed to ascertain whether treating vaginal dysbiosis in asymptomatic pregnant women will have a meaningful impact on the onset of premature labor. The study highlights the common vaginal microbiome present during the time of delivery and its effect on maternal and neonatal health outcomes. The study suggests imparting awareness to healthcare professionals and all women of reproductive age groups regarding the importance of maintaining a healthy vaginal microbiome.

ACKNOWLEDGEMENT

I express my heartfelt gratitude to my Guide and Co-guide for their guidance in conducting the study. I also sincerely thank Dr. Ramesh S. Paranjape, In-Charge Director, and all the faculty of Dr. Prabhakar Kore's Basic Science Research Laboratory, KAHER, Belagavi for the support and cooperation during the study. My acknowledgment will never be complete without thanking my study participants who actively participated in this study.

References

1. *Faruqui A. Bacterial Vaginosis: Risk of Adverse Pregnancy Outcome. J Gynecol Res Obstet. 2018; 4(2): 015-017. Available from: http://dx.doi.org/10.17352/jgro.000051*
2. *Khoudia D et al. Exhaustive repertoire of human vaginal microbiota. Human Microbiome Journal 11 (2019) 100051.*
3. *ShirleyGreenbaumMD et.al. Ecological dynamics of the vaginal microbiome in relation to health and disease. American Journal of Obstetrics and Gynecology. April 2019; 220(4), p324-335*
4. *Yang EV, Glaser R. Stress-induced immunomodulation and the implications for health. Int Immunopharmacol. 2002; 2:315–24. [PubMed: 11811934]*

5. *Gregory KE. Microbiome aspects of perinatal and neonatal health. The Journal of perinatal & neonatal nursing. 2011; 25:158–62. [PubMed: 21540692]*
6. *Kaambo E, Africa C, Chambuso R, Passmore J-AS. Vaginal Microbiomes associated with aerobic vaginitis and bacterial vaginosis. Front. Public Health. 2018. 6:78. Doi. 10.3389/fpubh.2018.00078*
7. *Shimaoka M.et al. Association between preterm delivery and bacterial vaginosis with or without treatment. Scientific reports.2019.9:509. DOI: 10.1038/s41598-018-36964-2*
8. *Freitas, A. C. et al. The vaginal microbiome of pregnant women is less rich and diverse, with lower prevalence of Mollicutes, compared to non-pregnant women. 2017; Sci. Rep. 7, 9212 Available from: https://doi.org/10.1038/s41598-017-07790-9.*
9. *Dingens et al. BMC Pregnancy and Childbirth. 2016; 16:278. DOI 10.1186/s12884-016-1073-y*
10. *Ravel J, Gajera P, Abdob Z, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A. 2011; 108:4680–7. [PubMed: 20534435]*
11. *Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in h uman body habitats across space and time. Science. 2009; 326:1694–7. [PubMed: 19892944]*
12. *Aderoba AK, Olokor OE, Olagbuji BN, Ande AB,Okonkwo CA, Ojide CK. Bacterial vaginosis in spontaneous preterm and term birth: A case–control study. Trop J Obstet Gynaecol 2016;33:297-301*
13. *Bosede B. Afolabi, 1 Olusanjo E. Moses, 2 and Oyinlola O. Oduyebo3. Bacterial Vaginosis and Pregnancy Outcome in Lagos, Nigeria. Open Forum Infectious Diseases. 2016; 1-5*
14. *Brocklehurst P, Gordon A, Heatley E, Milan SJ. Antibiotics for treating bacterial vaginosis in pregnancy. Cochrane Database Syst Rev 2013; 1:CD000262.*
15. *Vaginal microbiome in late pregnancy and parturition. ISRCTN Registry. BMC. https://doi.org/10.1186/ISRCTN29892347*
16. *Chopra C, Bhushan I, Mehta M, Koushal T, Gupta A, Sharma S, Kumar M, Khodor SA, Sharma S. Vaginal microbiome: considerations for reproductive health. Future Microbiol. 2022 Dec;17:1501-1513. doi: 10.2217/fmb-2022-0112. Epub 2022 Oct 31. PMID: 36314380.*
17. *Serrano MG, Parikh HI, Brooks JP, et al.. Racioethnic diversity in the dynamics of the vaginal microbiome during pregnancy. Nat Med 2019; 25: 1001–1011.*
18. *Arena B, Daccò MD. Evaluation of vaginal microbiota in women admitted to the hospital for premature labour. Acta Biomed. 2021 Nov 3;92(5):e2021292. doi: 10.23750/abm.v92i5.9925. PMID: 34738595; PMCID: PMC8689340.*
19. *Sakabe Y, Nishizawa H, Kato A, Noda Y, Ohwaki A, Yoshizawa H, Kato T, Sekiya T, Fujii T, Kurahashi H. Longitudinal study of the vaginal microbiome in pregnancies involving preterm labor. Fujita Med J. 2022 Aug;8(3):96-101. doi: 10.20407/fmj.2021-017. Epub 2021 Nov 25. PMID: 35949516; PMCID: PMC9358670.*
20. *Dan Li, Xin-Zuo Chi, Lei Zhang, Rui Chen, Jing-rong Cao, Xiao-yan Sun, He-qin Yang, Qin-ping Liao; Vaginal microbiome analysis of healthy women during different periods of gestation. Biosci Rep 31 July 2020; 40 (7): BSR20201766. doi: https://doi.org/10.1042/BSR20201766*
21. *Aslam S, Sayeed Saraf V, Saleem S, Saeed S, Javed S, Junjua M, Bokhari H. Lactobacillus species signature in association with term and preterm births from low-income group of Pakistan. J Matern Fetal Neonatal Med. 2022 Aug;35(15):2843-2852. doi: 10.1080/14767058.2020.1810660. Epub 2020 Sep 6. PMID: 32892671.*