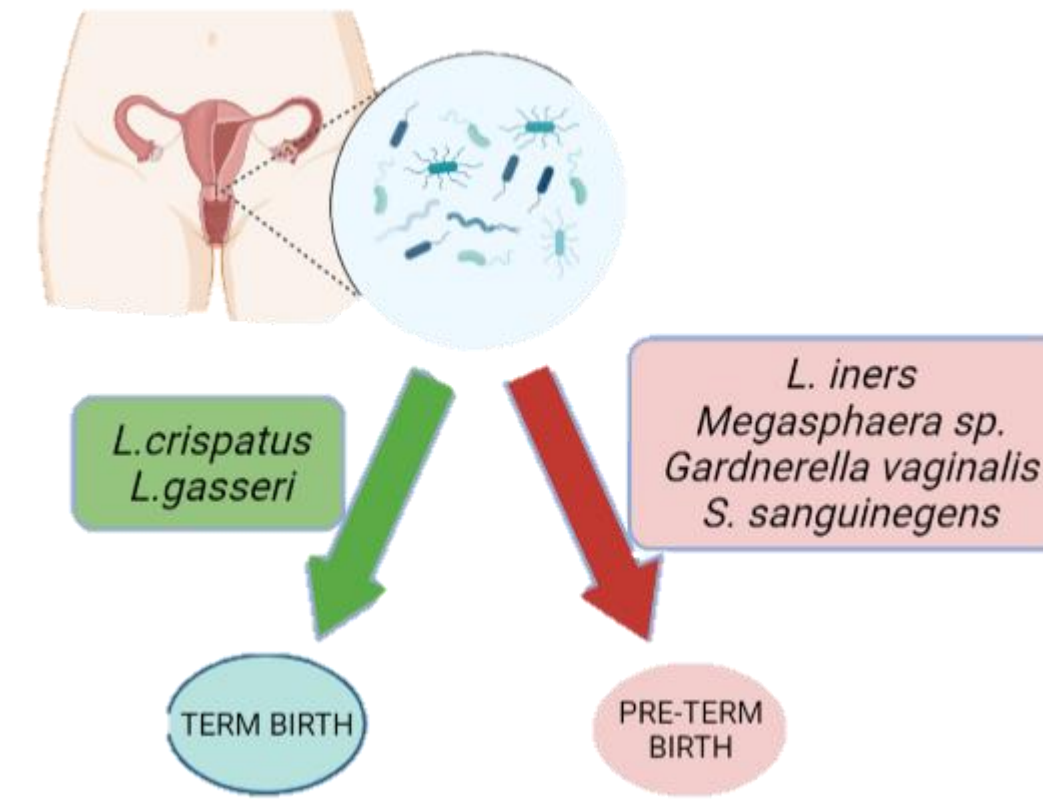


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## INTRODUCTION

Preterm births (PTB) account for approximately 13% of all births in India. PTB is a multifactorial disease, and microbial communities or specific taxa living in the vaginal milieu of pregnant women contribute significantly to birth outcomes. Thus, investigating the diversity, dynamics, and functions of the vaginal microbiome of women delivering term and preterm babies is critical.

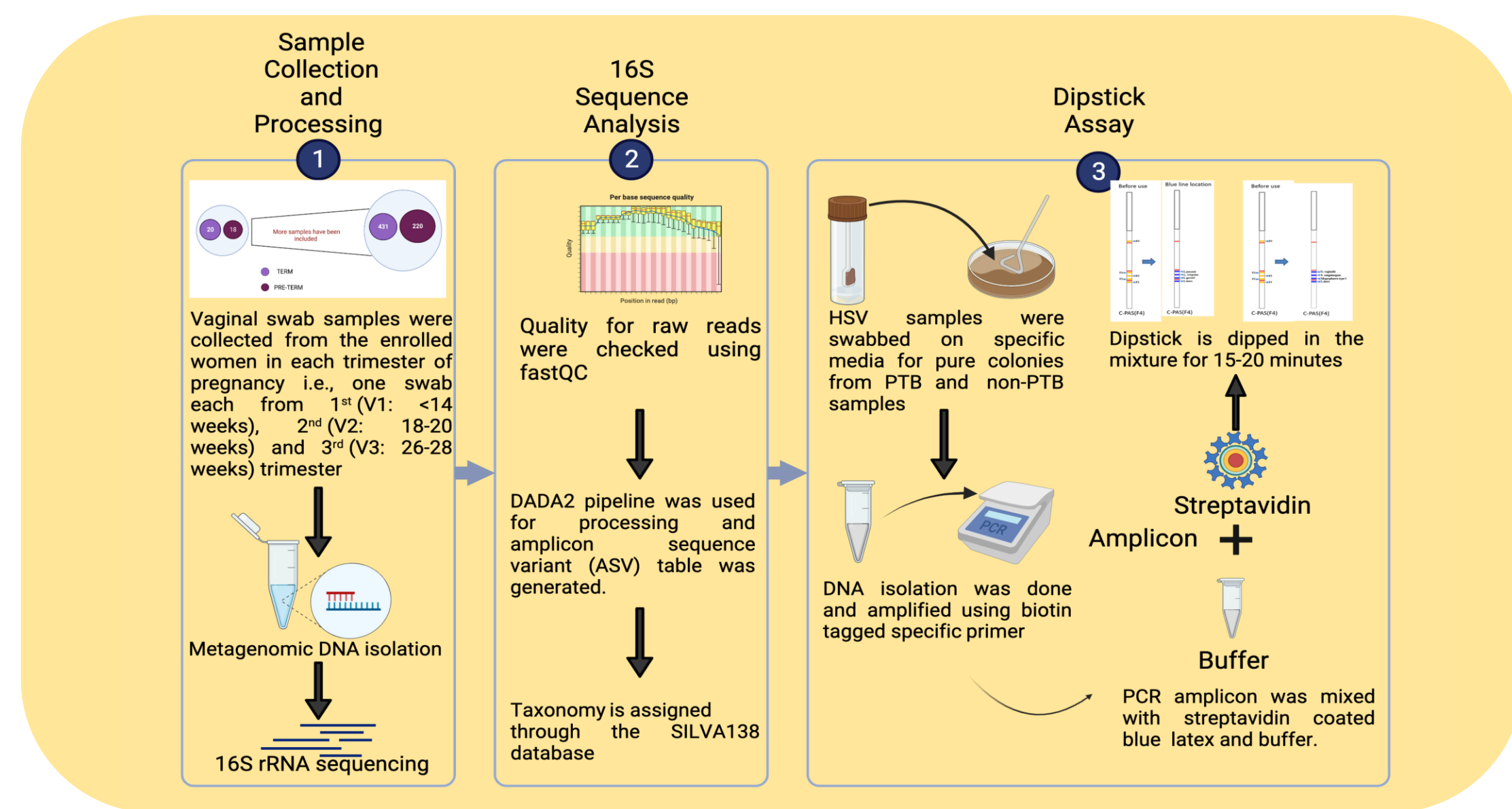


## OBJECTIVE

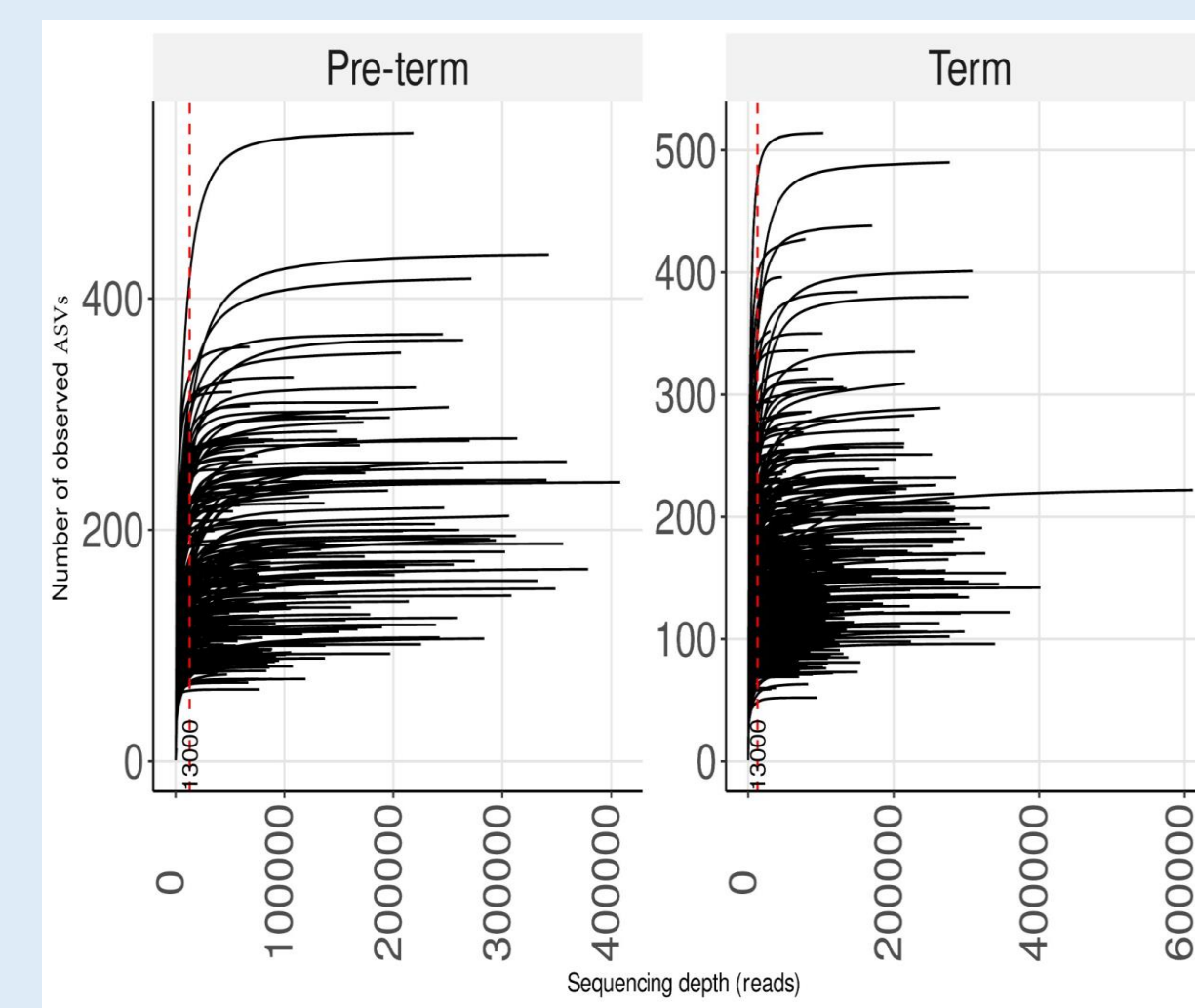
This longitudinal study was done to validate our previous finding of dysbiosis in the vaginal microbial flora in term and preterm deliveries and to develop a diagnostic tool for the rapid and early detection of risk factor for preterm birth.

## METHODS

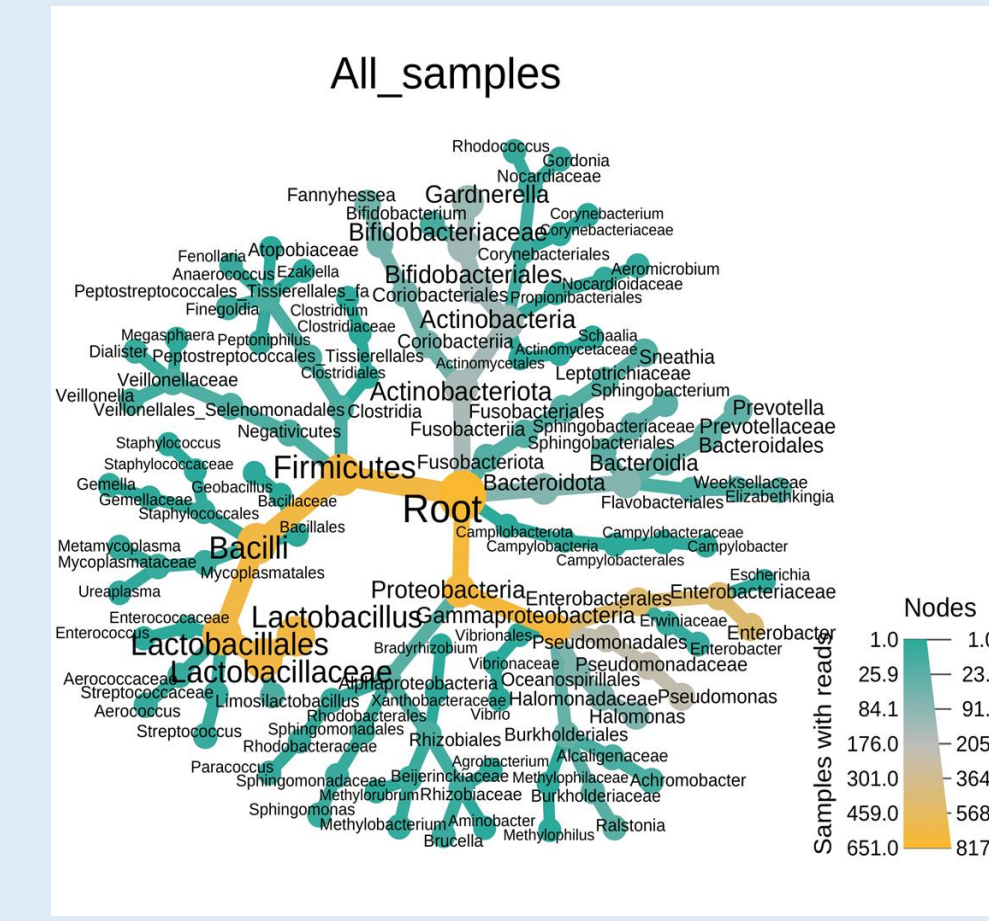
- Samples were collected longitudinally at three time points (V1, V2, and V3),
- Metagenomic DNA isolation and 16S rRNA gene sequencing were performed to identify the vaginal microbiome,
- PICRUSt was used to predict the functional content of the metagenome,
- A dipstick assay was performed to develop a diagnostic tool for the early prediction of preterm birth.



## RESULT



Rarefaction curve of term and pre-term birth shows that after 13000 reads very less increment of ASV is observed.



Taxonomy of all microbes found in HVS samples

	Pre Term			Term		
Lactobacillus iners	40447	23894	22704	24201	16920	17631
Lactobacillus crispatus	11110	19172	17988	22429	19164	20336
Lactobacillus gasseri	255	275	255	504	1499	732
Lactobacillus jensenii	90	32	25	400	796	848
Lactobacillus delbrueckii	1062	486	707	123	301	196
Lactobacillus johnsonii	0	0	0	335	450	274
Lactobacillus reuteri	114	110	113	94	97	90
Lactobacillus delbrueckii	2	13	6	55	22	20
Lactobacillus acidophilus	8	21	21	29	9	10
Lactobacillus colohominis	17	11	5	9	8	6

Trimester wise read count distribution of top 10 Lactobacillus sp. in term and pre-term group. preterm deliveries had a higher proportion of L. iners (32% vs. 21.67% in term birth)

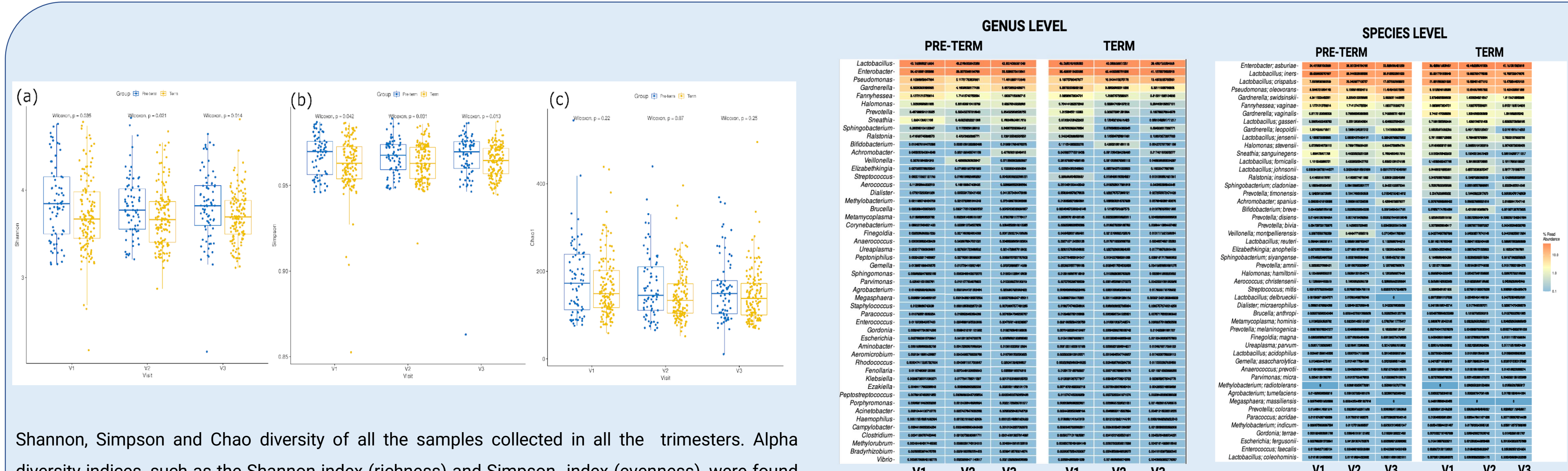
## DISCUSSION

In the present study, *L. crispatus* and *L. gasseri* have been commonly found to be associated with term birth outcomes. These organisms produce lactic acid and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which maintain an acidic pH, and the secretion of ribosomally encoded antimicrobial peptides that provide protection to the vaginal milieu against the colonization and growth of exogenous and potentially harmful bacterial taxa.

## CONCLUSION

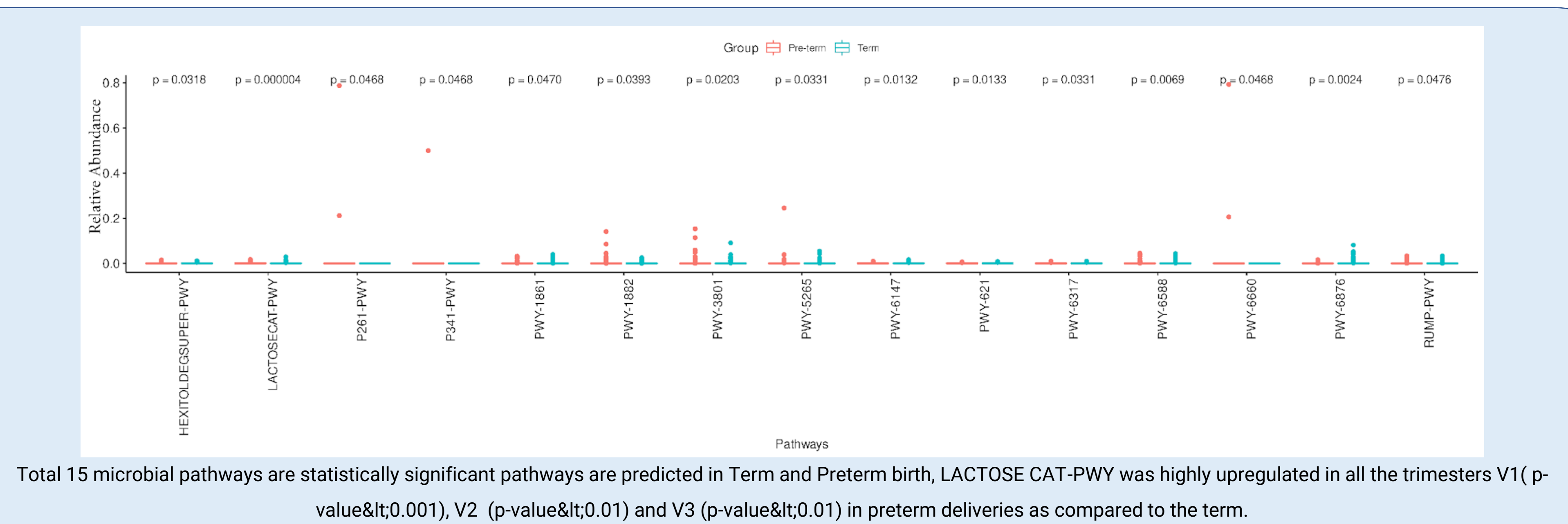
- The current study indicates that birth outcomes are correlated with the dominance of particular Lactobacillus species and a few other facultative anaerobes.
- *L. crispatus* was found to be dominant in the vaginal milieu of mothers with term birth outcomes. Thus, it can be considered a potent candidate for the development of probiotics.
- It was found that term birth (16.6%) had a higher abundance of *L. crispatus* than preterm deliveries (13%). In preterm birth, some anaerobic pathogens like Gardnerella, Sneathia, Prevotella, Aerococcus, Veillonella were found to be more abundant.

Shannon, Simpson and Chao diversity of all the samples collected in all the trimesters. Alpha diversity indices, such as the Shannon index (richness) and Simpson index (evenness), were found to be statistically significant between term and preterm delivering mothers in all the trimesters.

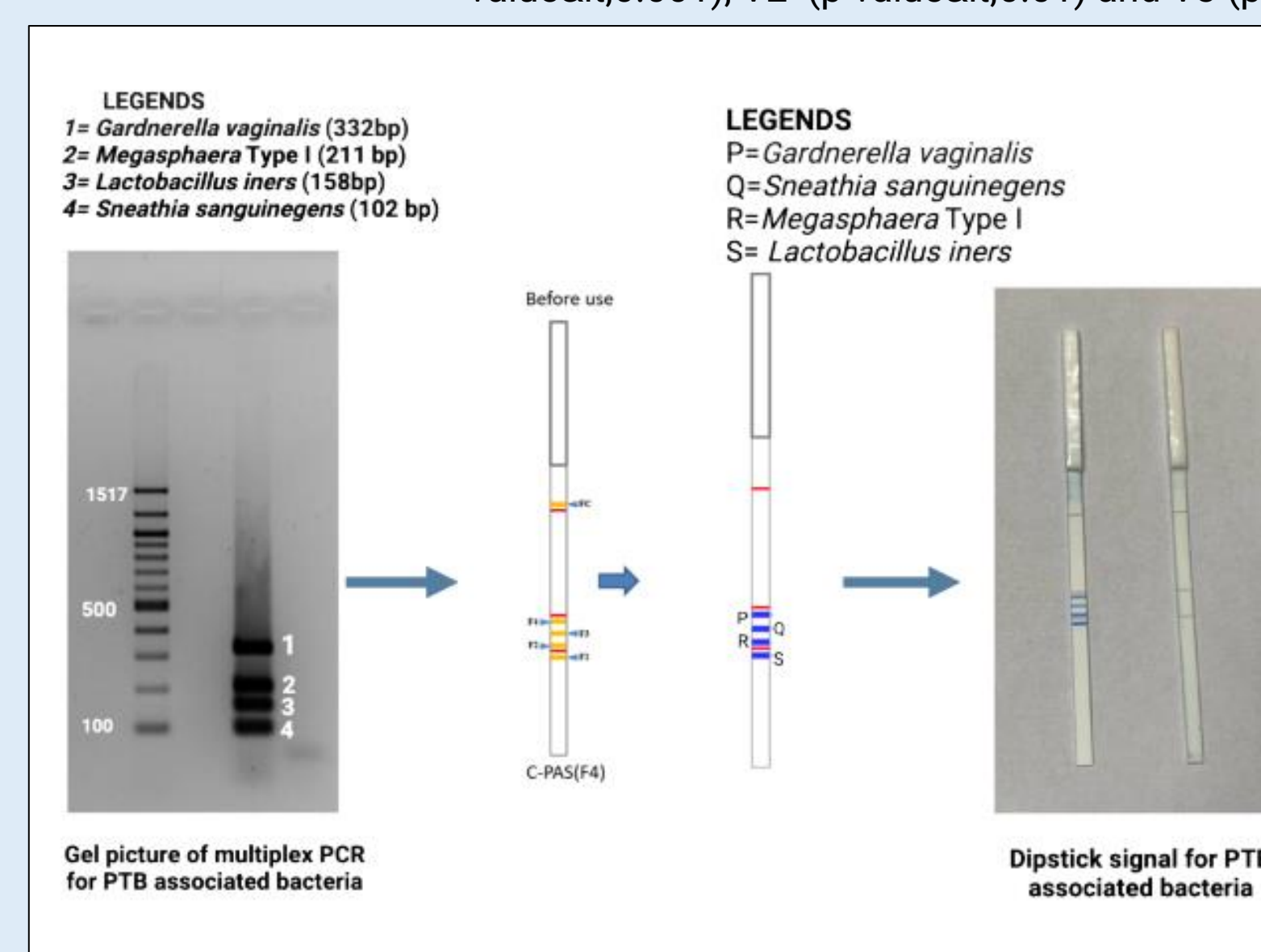


Relative abundance of top 50 Genus in V1, V2, and V3 trimesters of term and pre-term group (based on 40% core OTU).

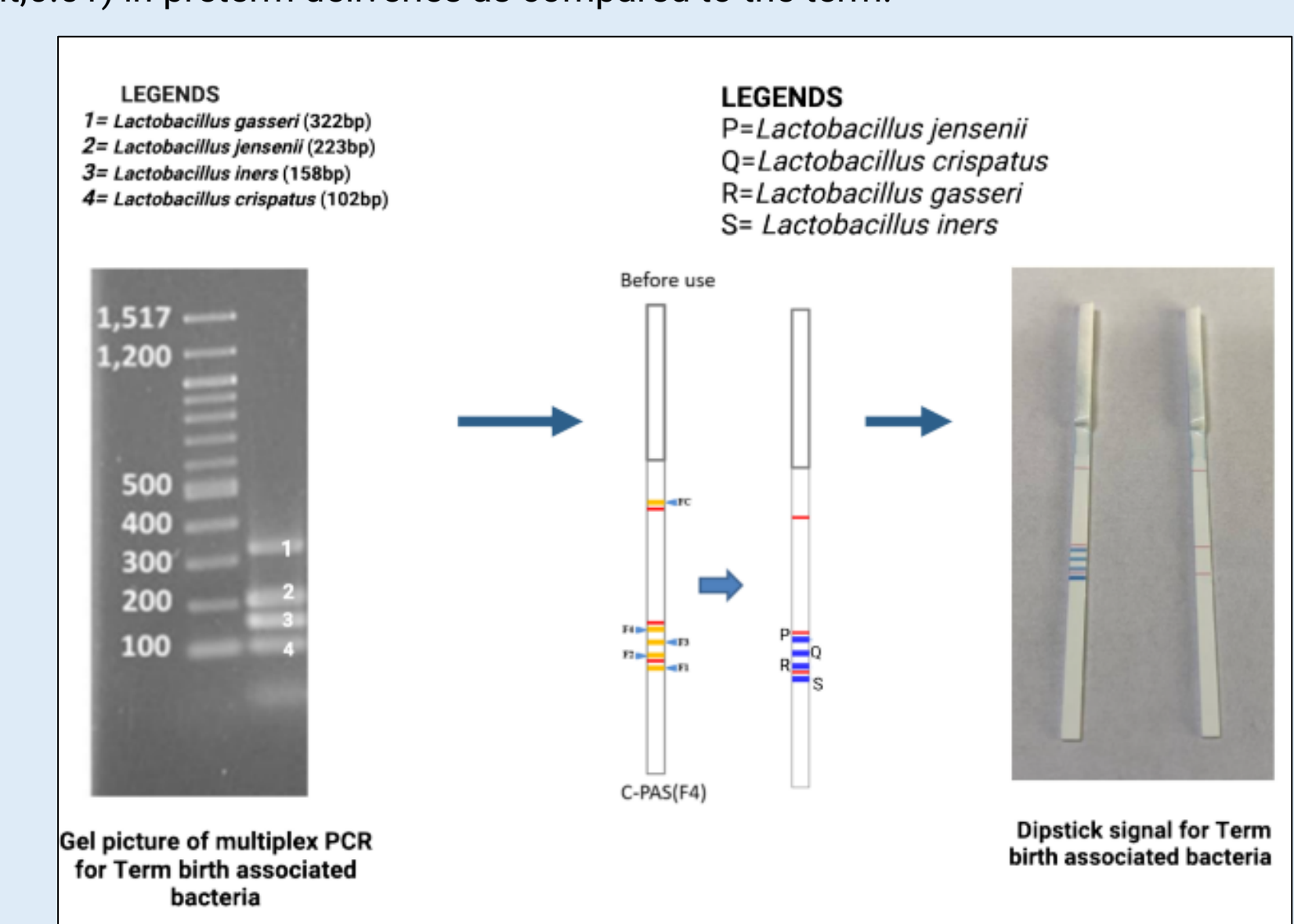
Relative abundance of top 50 species. Each ASV was identified based on BLASTn 16S rRNA database of NCBI



Total 15 microbial pathways are statistically significant pathways are predicted in Term and Preterm birth, LACTOSE CAT-PWY was highly upregulated in all the trimesters V1 (p-value<math><math>0.001</math>), V2 (p-value<math><math>0.01</math>) and V3 (p-value<math><math>0.01</math>) in preterm deliveries as compared to the term.



Dipstick assay for pre-term and term birth for seven bacterial species associated with pre-term and term deliveries



Our uniplex and multiplex PCR assays followed by the dipstick-based detection precisely identified the presence of Gardnerella, Sneathia, Megasphaera, *L. iners*, *L. crispatus*, *L. gasseri* and *L. jensenii* in the HVS samples of pregnant Indian women.

**KEY MESSAGE:** Developing a potential probiotic to maintain a healthy vaginal microbiome environment can help control preterm birth. The rapid detection of microbial communities through a dipstick assay can help in the early diagnosis of preterm delivery.

## ACKNOWLEDGEMENT

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