

VAGINAL MICROBIOME SIGNATURES ASSOCIATED WITH PRETERM BIRTH

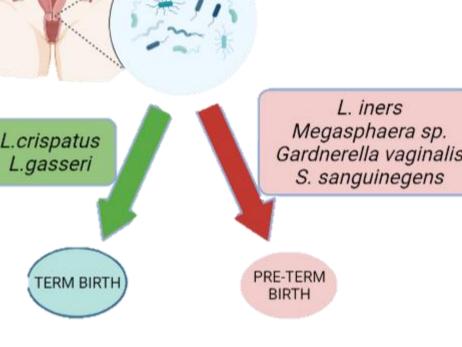
<u>**Taruna Ahrodia,</u>** Shakti Kumar, Daizee Talukdar, Souvik Mukherjee, GARBH-INI study group, Bhabatosh Das *Functional Genomics Laboratory*</u>



Translational Health Science and Technology Institute

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.

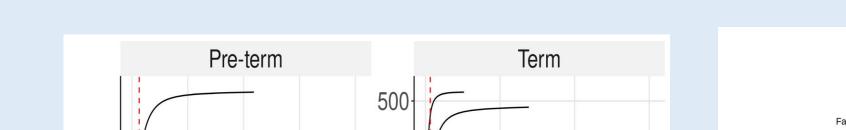


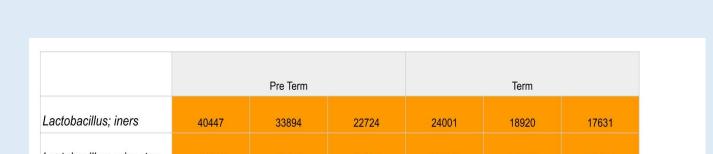
OBJECTIVE

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

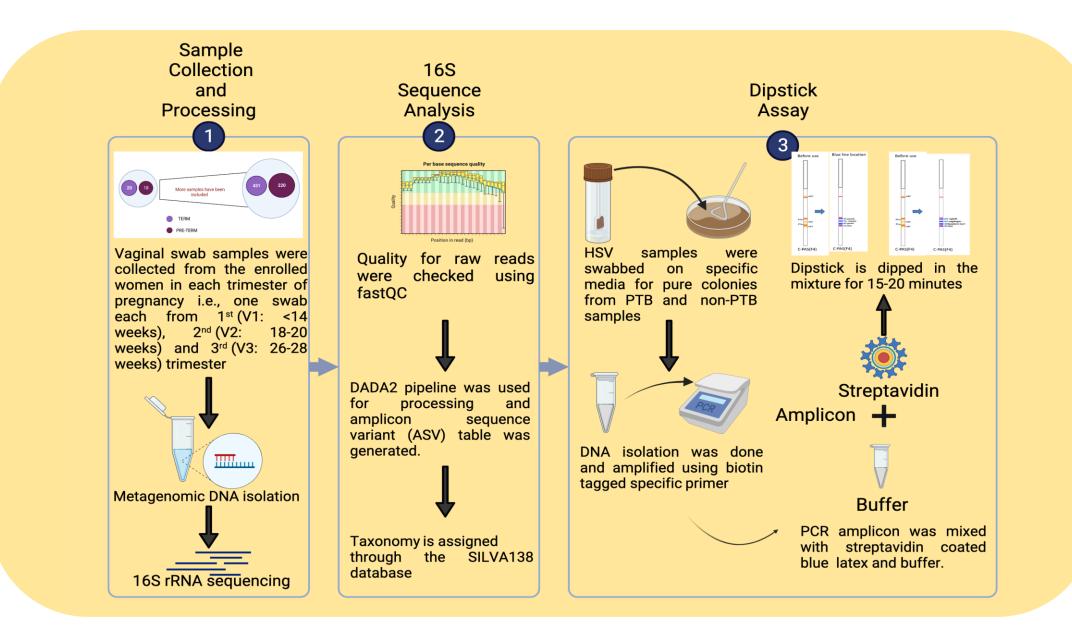
• Samples were collected longitudinally at three time points

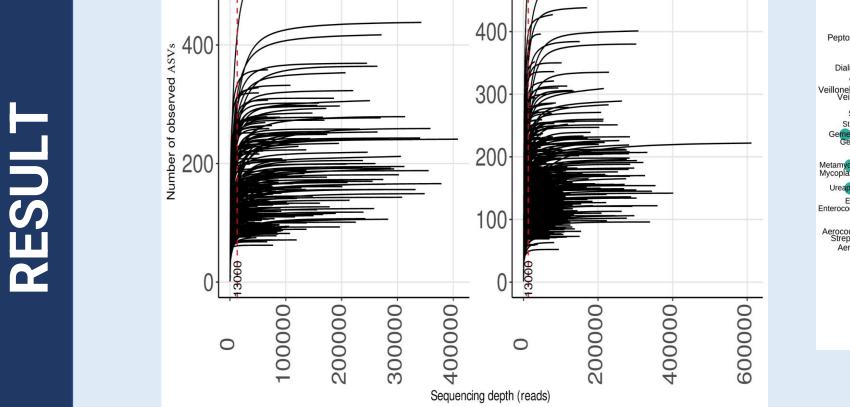




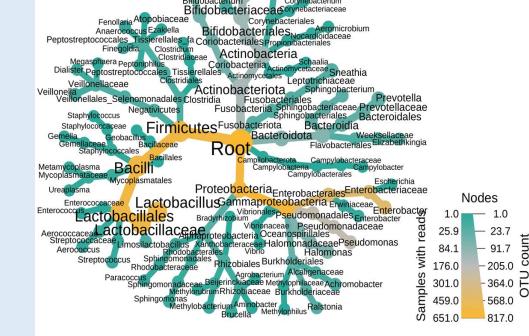
(V1, V2, and V3),

- 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.





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

All samples

Lactobacillus; crispatus	11110	19172	17668	22429	19164	20336	
Lactobacillus; gasseri	255	275	255	504	1499	732	
Lactobacillus; jensenii	90	32	25	400	706	848	
Lactobacillus; fornicalis	1082	486	707	123	301	196	% Read Abundar
Lactobacillus; johnsonii	0	0	0	335	450	274	10
Lactobacillus; reuteri	114	110	113	94	97	90	10
Lactobacillus; delbrueckii	2	13	0	55	22	20	0.
Lactobacillus; acidophilus	8	21	21	29	9	10	
Lactobacillus; coleohominis	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)

										Lactobacillus	
										Enterobacte	
										Pseudomona	
(a)	Group 逹 Pre-term 逹 Term		(h)	Group 幸 Pre-term 逹 Term		(a)	Group 喜 Pre-term 🝺 Term		Gardnerella Fannyhessea	
	a)			(b)			(C)			Halomona	as- 1.009098
	Wikoxon, p = 0.035	Wilcoxon, p = 0.021	Wilcoxon, p = 0.014	Wilcoxon, p = 0.042	Wicoxon, p = 0.031	Wilcoxon, p = 0.013	Wilcoxon, p = 0.22	Wilcoxon, p = 0.07	Wilcoxon, p = 0.25	Prevotella	
	•r i		•			•t 1	eritovoli, p = 0.22	Widewon, p = 0.07	1000001, p = 0.60	Sneathia Sphingobacteriun	
		· · · ·	t			-21				Ralstonia	
		l;	3.1						•	Bifidobacteriun	
		• • •				191				Achromobacte Veillonella	
		· · · · · · · · · · · · · · · · · · ·					•.	•		Elizabethkingia	
							1.			Streptococcus	US- 0.0822710
4				0.95	4 C 👯		400-	•		Aerococcus	US - 0.112898
						• •			N	Dialiste Methylobacteriun	
								· · · · · ·		Brucella	
										Metamycoplasma	1a - 0.013082
LOL			- Tr 🔣 🕹	Los			5		and the second second	Corynebacteriun	m - 0.000215
Shannon				d			Chao	•	1	Finegoldia Anaerococcu	JS- 0.000000
S				ω υ					* * *	Ureaplasma	na - 0.0000179
			N	•						Peptoniphilus	
			• •	0.90			200-			Gemella Sphingomona:	
3	3-						1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1			Parvimonas	as- 0.0254015
		• •						1 - A - A - A - A - A - A - A - A - A -		Agrobacteriun	m- 0.01450250
										Megasphaera	
										Staphylococcu: Paracoccu:	
								1 No. 787		Enterococcus	US- 0.0116230
									• • •	Gordonia	ia- 0.00024677
										Escherichia Aminobacte	
	•		•	0.85-						Aeromicrobium	
		No	va		via la	10	01			Rhodococcus	US- 0.000047411
	V1	V2 Visit	∨3	V1	V2 Visit	V3	V1	v2 Visit	V3	Fenollaria	
								VISIC		Klebsiella Ezakiella	
										Ezunione	-

GENUS LEVEL							SPECIES LEVEL							
								ər	COIE2	LEVEL				
PRE-TERM TERM						PRE-TERM TERM								
								PRE-IERIVI			TERM			
Lactobacillus-	48.1529090216604	46.2766000643388	43.0374085381048	46.0680767608383	40.000530017357	38.4867153384666	Enterobacter; asburiae-	34.4018081063686	38.3073348194768	33.525056421238	25.4226615838487	42.4462296761835	41.1673073023818	
Enterobacter-	34,4218501053508	39.3073348194796	33.8286373419641	35.4590813420085	42, 445/20057511250	41.1873572622915	Lactobacillus; iners-	32.6290639767697	25.0443228085836	25.0130522061522	20.8217791838049	10.0027034770058	16.7587223476676	
Pseudomonas- Gardnerella-	6.1030505047994	5.11701783832001 4.16069083177436	11.4010002110040 5.05700002498671	6. 19279795497677 6. 29732103952139	10.8434418076178	13.4423036765562	Lactobacillus; crispatus-	7.63299080963509	29.0403977198787	17.0078002900928	21.0010063001930	18.6594814871013	18.4759544620108	
Fannyhessea-	3.1272121579814	1.71415742752584	1.08387150080716	2.08580973834701	1.20857575588021	0.016511925134906	Pseudomonas; oleovorans-	8.09467210694162	6.10930108324016	11.4542406073296	8, 15125246198586	10.6545670067382	13.4554000091000	
Halomonas-	1.00306673651805	0.831626710416769	0.62676545222555	0.704141262572548	0.325847439457212	0.204402450807101	Gardnerella; swidsinskii-	4.94115224452551	8.20543133059966	3.99290511448695	2.67345425566638	1.40082945216547	1.01154314002900	
Prevotella-	0.457206591015025	0.323452737218943	1.85452500538753	1.3153965110383	0.329273901281894	0.182736078546278	Fannyhessea; vaginae-	3.12721213759614	1.71413742755584	1.08337150080715	2.06586673834701	1.20837676569621	0.815311835134808	
Sneathia-	1.8684736877798	0.460233202201008	0.700940534017015	0.612094236422503	0.130402124676423	0.0001042001711217	Gardnerella; vaginalis- Lactobacillus; gasseri-	0.071731206365306	0.55712505040804	9.745989978142816 0.424982255942041	2.74451470399966	1.80842969080899	1.331004253242	
hingobacterium-	0.202500104122047	0.11725059128012	0.349675550964412	0.007050302678534	1.0783905304382345	0.3345308517289771	Gardnerella; leopoldii-	1,20742656718611	0.100041040001312	1.11416083638034	0.890306815362266	0.447173200120807	0.077816916714203	
Ralstonia-	0.416262745898573	0.42023906398771	0.239135540502552	0.546345208058299	0.125504735981262	0.106023573027283	Lactobacillus; jensenii-	1.10838720323898	E.0808045754804101	0.0254267950279502	0.7711538712585	0.76048575753554	C. 7762221579625725	
Bifidobacterium-	0.0104676164470366	0.00351591252593495	0.0196817954976375	0.111054255222278	0.425021001001118	D.0542757977951189	Halomonas: stevensii-	0.672566246736113	0.7390176994091	0.504457598794794	0.014549620151999	0.200700101362010	A 207420736209458	
Achromobacter-	0.0405052943844948	0.0001080456741109	0.427928319249415	0.0429827718318436	0.0012304304020400	0.217401920625077	Sneathia; sanguinegens-	1.6591736577758	0.450235215201098	0.756945594617816	0.612024539422603	0.130402124575425	9.000104201711217	
Veillonella-	0.05701094924248	0.4905250300002647	0.07130630022939097	0.0016793674696165	0.00100300763031112	0.0408086888094287	Lactobacillus; fornicalis-	1.10150482000721	0.420283253457703	0.005531301074100	0.148385422407788	0.50120038705805	D. 191170006180297	
Elizabethkingia-	0.0270355738053341	0.0718531857581963	0.152030048054894	0.029054352548643	0.0857342751322822	0.102234770677061	Lactobacillus; johnsonii-	0.0000843957301448271	0.000304808185832898	0.000127275745462661	0.844080019883881	0.455738360825347	0.201717815067272	
Streptococcus-	0.0822710521127756	0.0196109825680267	0.00483006232569721	0.2000/04000537	0.0100408780304827	0.0131083667627361	Ralstonia; insidiosa-	0.41450516179781	0.4160867746711882	0.238091225240988	9.046763867652251	0.124970280362009	D. 104200228320008	
Aerococcus-	0.11205064232519	0.105189567400495	0.0200002525300504	0.0310431504443049	9.0182528017381818	0.042952590943445	Sphingobacterium; cladoniae -	0.155094850534085	0.0641380853801777	0.944221023673344	0.505078022955068	0.0551085078888951	0.232234853014546	
Dialister-	0.070015203081029	0.0355591700431482	0.0413373484473086	0.0500949679270633	0.0292707573000121	0.0272656872474428	Prevotella; timonensis-	0.124230106735085	0.104174889060658	0.0183421554214612	0.30476308860832	0.104408223017673	0.0830804791742859	
ethylobacterium-	0.0211950745454709	0.0210720801044348	0.0754860780063566	0.0130626272690891	0.00000000000000000	D. 05780-8086190676	Achromobacter; spanius-	0.0360304318108386	0.0863618072255336	0.425646726372077	0.0379763689966422	0.0003270000221610	D.216800417047148	
Brucella-	0.00838944598229823	0.00021725123362532	0.0025263639062957	0.000404675360046148	0.121007020007073	0.0102700025901895	Bifidobacterium; breve-	0.0044085851554192	0.002089222895452285	0.0001348545417161	0.0705971747054804	0.421095190959979	0.0510671267878525	
etamycoplasma-	0.013962963529782	0.00230614086161087	0.0790799117778417	0.0659979180483195	0.00232396006620311	0.0040325355555555000	Prevotella; disiens-	0.0142481367694864	0.007748194082855	0.000302704406106049	0.620660085018189	0.0307235594047948	0.008206772482547684	
orynebacterium-	0.0000215434531453	0.0220810724527859	0.00640525810015385	0.029259882000086	0.0139278359198782	0.00084410804487482	Prevotella; bivia-	0.0347267221752675	0.14290337228403	0.00943802034154596	0.307389538049417	0.0367857786672327	0.0404352949026785	
Finegoldia-	0.02652963668657226	0.0271990824904099	0.00812902784708686	0.0450425001050401	9.0212796920702679	0.0131111521098084	Veillonella; montpellierensis-	0.000725562780359	0.484847718585218	0.0713409417952801	0.0405724857867969	0.0489095178743148	0.0440298359912994	% Read Abundance
Anaerococcus-	0.0303036520435409	0.0408978847001021	0.00495228959182904	0.0327107124390128	0.0179718329068702	0.0204057462135283	Lactobacillus; reuteri-	0.0004041000001014	D. 0596913097805407	9.112200007044210	0.0011421707033436	0.0004710020434488	0.000007000200000	Additioance
Ureaplasma-	0.0830171000000001	0.0276661725666502	0.0214729997918902	0.0210167636854632	0.0227028803884055	0.0177100783004450	Elizabethkingia; anophelis-	0.0270355736053241	0.0718631957501963	0.152030048054854	0.039654352549543	0.0857242751322822	0.16223477697681	10.0
Peptoniphilus-	0.0232422217465967	0.0277526138096267	0.00599737357767902	0.0407764833194547	9.019422708261089	0.00891617179960602	Sphingobacterium; siyangense -	0.0754052534007528	0.033219806888042	0.105054527321000	0.194083694004396	0.0232622825515294	0.091871992258205	
Gemella-	0.0130851899428575	0.0137294163601461	0.0202088885114089	0.0509422557799195	0.00384517654530858	0.00410050082001375	Prevotella; amnii- Halomonas; hamiltonii-	0.2222270696431	E.0810807022238947 E.0828915210548774	0.128029822982629	0.0000054042200455	0.0218438107748022	0.02597672021504276	1.0
Sphingomonas- Parvimonas-	0.025401531553781	0.0101577304878803	0.0122298279130019	0.0272785286788538	0.00514535861070272	0.03423201391953809	Aerococcus: christensenii-	0.112956944632519	0.1000000000000000		0.0535991420922565	0.016232046104582	0.0000000000000	1.0
Agrobacterium-	0.014362629420825	D.00010444131305454	0.020500752053422	0.00000000000000000	0.0051155505104653	0.0170666118708402	Streptococcus; mitis-	0.000157375220545201	0.00768579554795118	0.00203707675246579	0.229925421801905	5.00738101869078298	0.0000001430485479	
Megasphaera-	0.0096913434850167	0.000104880185872504	0.000072304247165011	0.5480807094170251	0.00111405021204154	0.000212421383648209	Lactobacillus; delbrueckii-	0.00158087100347571	0.0159004965799246		0.0927359912107839	0.0334624041495184	0.0407535248502091	0.1
Staphylococcus-	0.01230996745639	0.00910865329973168	0.00708997577481285	0.0198774748556804	0.00000000027200004	0.00057570748314259	Dialister: micraerophilus-	0.0000016790024305	E.0294642270569446	9.040226709002339	0.0481981880443714	0.01779490057071	0.0206774704306279	
Paracoccus-	0.0107925918050254	0.0192525450594059	0.307605473462592787	0.0132462730153868	0.0003007241220301	0.00767176550060648	Brucella; anthropi-	0.00020792363343484	0.0000442780015686859	0.0020255451257729	0.000437558640052989	0.121927045290013	0.0102700025001805	
Enterococcus-	0.0110230840267463	0.00349691625000996	0.004792814400096987	0.00010058594296759	0.0189618067048574	0.00990579198583598	Metamycoplasma; hominis-	0.013052563629782	0.00230814066161067	0.0780768117776417	0.0003070180453105	0.00230296006620311	0.00412525558885805	
Gordonia-	0.00824877243874298	0.00004515191121952	0.0182763946186806	0.00704220204815497	0.00094286278008742	0.010425591801787	Prevotella; melaninogenica-	0.00827800782247277	0.004000000000000000	0.160220598120497	0.00270404170370079	0.00438697508060846	0.0002774434225791005	
Escherichia-	0.00276822615708641	0.0412913074733378	0.0000002120000002	0.0134158078808211	0.00120089448658468	0.00193439308757883	Finegoldia; magna-	0.0283265898257225	0.0271000634804040	0.00012002734798595	0.0459438001989481	0.0212700820702079	0.0151111821008004	
Aminobacter-	0.0061600062632/30	0.00472263670586004	0.0139100250612994	0.00812211600510185	0.0036823122958448277	0.01346/6317066123	Ureaplasma; parvum-	0.0530171080606901	0.02786017238386602	0.0214720007010002	0.0200167636888882	0.0227020803624055	0.0177108799904499	
Aeromicrobium-	0.00213416901429607	0.00434962793229796	0.0167901532530625	0.00220030109109571	0.0019494567749957	0.0174205756205113	Lactobacillus; acidophilus-	0.00544613596140525	E.0305703471132088	0.0210420552031804	0.0227308043359854	0.0101538155405159	0.015596555560525	
Rhodococcus-	0.0000474112067327654	0.00430011617003667	0.026341324259807	0.0002296299666948625	0.00044007986204726	0.0110553367636668	Gemella; asaccharolytica-	0.013480844575181	0.0131401775841696	0.0202068895114089	0.0481057192382812	0.0021200903344329	0.003016163631375465	
Fenollaria-	0.0118746068120085	0.00754401225559943	0.005859193374819	0.0132017214207625407	0.00371807888978179	0.0011821682868288	Anaerococcus; prevotii -	0.0162486361448368	0.0340825636473601	0.00212734823130875	0.0228102808428748	0.0130199163661448	0.01401662283888274	
Klebsiella-	0.000967362212380571	0.0177941798211597	0.00121832968205253	0.0108381287077917	0.00545041728013703	0.0038258578240778	Parvimonas; micra-	0.025401531563791	0.0101577304878803	0.0122098279130018	0.0272785295786339	0.00514533801070272	0.004232015812533089	
Ezakiella-	0.00484117852298848	0.00200000000000000	0.00200211692101178	0.00716721628302715	0.00733432576060434	0.00426523148006626	Methylobacterium; radiotolerans-	0	0.00368183256778381	0.0529961507077768	0	0.00826563291524989	0.018565067865817	
tostreptococcus-	0.00789197483931982	0.000666603467056604	0.000334003762859486	0.0110747450308359	0.00372255241671074	0.00236488398388228	Agrobacterium; tumefaciens-	0.0148205065952616	0.009/190723254861079	0.0200007920000422	0.00830273420546182	0.0062567947081469	0.0170510246444864	
Porphyromonas-	0.00000010463635268	0.00154386446666664	0.00221729699781977	0.0000000000000000000000000000000000000	0.005696672285021051	9.00140206167560975	Megasphaera; massiliensis-	0.02975495519525999	0.0000425344581207854	0	0.0443159500443455	0	8	
Acinetobacter-	0.00310444130718773	0.00574376679232562	0.00583258483748709	0.00244389520008194	0.00408030116237864	0.00481316226519555	Prevotella; colorans- Paracoccus: acridae-	0.0164994129381874	0.00239041622911689	0.00900984113963868	0.0259804125466298	0.006366394846466827	0.00298231729469911	
Haemophilus-	0.000773378287632394	0.00102731652142835	0.000120740001623620	0.0186987747541818	0.001210/252/744701	0.0008789828283343	Paracoccus; acridae-	0.01010/420/163629	0.017003101448578	0.00/30028/8040148	0.013040004412361	202344/04110/1628	0.00710400/4254438	

DISCUSSION

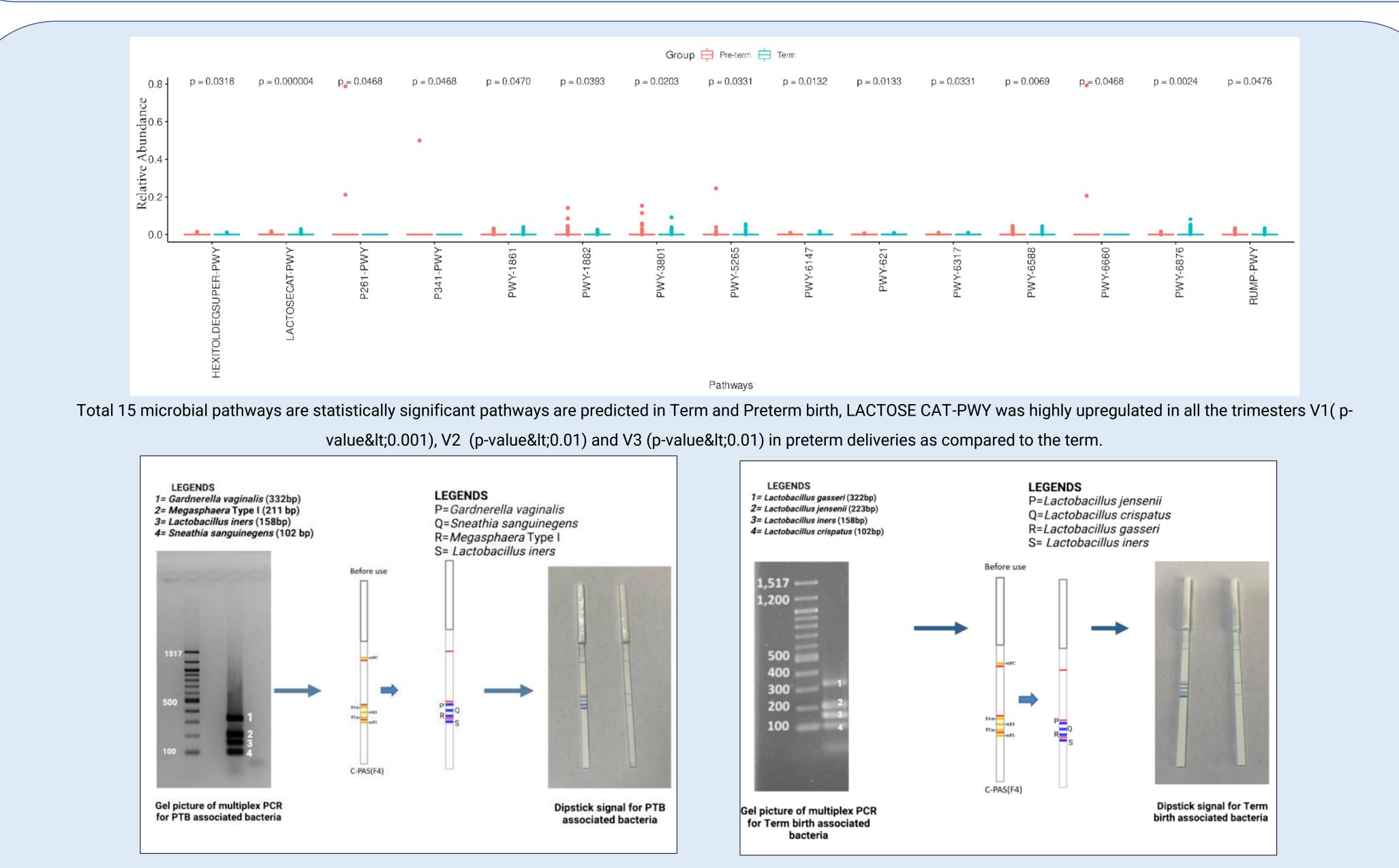
In the present study, L. crispatus and L. gasseri have been commonly found to be associated with term birth outcomes. These organisms produce lactic acid and hydrogen peroxide (H2O2), which maintain an acidic pH, and the secretion of ribosomally encoded antimicrobial peptides that provide protection 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. 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,	Relative abundance of top 50 species. Each
and V3 trimesters of term and pre-term group	ASV was identified based on BLASTn 16S
(based on 40% core OTU).	rRNA database of NCBI



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.

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, Megasphera, 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.



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