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Vaginal and amniotic fluid microbiota in women with preterm labor – a comparative microbiological study

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ABSTRACT

Introduction and aim. This observational comparative study characterized the vaginal and amniotic fluids of women in preterm labor relative to those who delivered at term. A distinctive feature of this work is the paired, quantitative and culture-based assessment of both the vaginal and the amniotic fluid microbiota, while most previous studies have examined the vaginal compartment alone.

Material and methods. Carried out at a single tertiary center, the study enrolled 120 women in preterm labor at 33–35 weeks of gestation together with 89 women delivering at term (≥38 weeks). Culture-based identification and quantitative analysis were applied to paired vaginal and amniotic fluid specimens.

Results. Preterm labor was accompanied by a reduced prevalence of *Lactobacillus* spp. and by a markedly higher recovery of *Gardnerella vaginalis* (60.8%; odds ratio [OR] 12.27, 95% confidence interval [CI] 5.78–26.06) and *Ureaplasma* spp. (45.8%; OR 9.91, 95% CI 4.23–23.22), exceeding the term group ($p<0.001$). Higher vaginal pH and polymicrobial associations occurred more often. The amniotic fluid produced microbial growth in 53.3% of preterm cases and vaginal and amniotic detection of *Ureaplasma* spp. ($\varphi=0.61$; $p<0.001$).

Conclusion. Vaginal dysbiosis showed an association with preterm labor that may be compatible with a contribution from ascending microbial colonization, though causality cannot be inferred from the present data.

Keywords. amniotic fluid, bacterial vaginosis, *Gardnerella vaginalis*, preterm labor, *Ureaplasma* spp., vaginal microbiota

Introduction

Preterm labor continues to be among the most prevalent problems in contemporary obstetrics, carrying an increased burden of perinatal morbidity and mortality along with lasting health sequelae in the newborn.[1-3] Its etiology is multifactorial, arising from an intricate interplay of genetic, environmental, and infectious contributors.

Previous work has shown that women with complicated pregnancies, especially those in preterm labor, frequently display shifts in the microbial makeup of the lower genital tract4,5. Such vaginal microbial changes can shape the local immune milieu and ease the upward passage of organisms from the lower genital tract into the uterine cavity.

A growing body of evidence links perturbation of the vaginal microbial ecosystem to unfavorable pregnancy outcomes. Overgrowth of opportunistic organisms can undermine the colonization resistance conferred by *Lactobacillus* spp., raise the vaginal pH, and reshape local immune responses.[6-10] Together, these shifts may promote migration of organisms toward the fetal membranes and amniotic cavity.

Infections of the genital tract have long been considered an important contributor to premature labor.[11-13] Bacterial vaginosis and nonspecific vaginitis are commonly documented among affected women.[14-18] Such conditions typically feature the depletion of protective *Lactobacillus* spp. accompanied by the expansion of anaerobic and opportunistic organisms.

Among these microorganisms, *Gardnerella vaginalis* and *Ureaplasma* spp. have attracted particular interest. Molecular microbiome surveys indicate that these organisms are often recovered in preterm labor and may participate in inflammatory processes on the fetal membranes.[19,20]

Recent studies have also described microbial presence within the amniotic fluid when pregnancy outcomes are adverse.[21-24] Although how organisms reach the amniotic cavity is still being clarified, ascending colonization from the lower genital tract is considered a plausible route.

Most current investigations of the vaginal microbiome depend on molecular assays that detect microbial DNA. Culture-based microbiology nevertheless retains clinical value, since it identifies viable organisms and quantifies the microbial burden and can therefore add complementary insight into the microbiological features linked with preterm labor. Quantitative culture data for the amniotic compartment and studies that sample the vaginal and amniotic microbiota in parallel remain comparatively scarce, leaving a gap that culture-based methods are well positioned to address.

Aim

This study aimed to characterize, qualitatively and quantitatively, the vaginal and amniotic fluids of women in preterm labor by culture-based microbiology, and to examine how these are related to microbial presence in the amniotic fluid. When combining quantitative vaginal and amniotic fluid cultures within a single

cohort, the study offers a microbiological perspective that complements the molecular microbiome analyses reported in our earlier work.

Material and methods

Study design and participants

The cohort comprised 120 pregnant women in preterm labor (Group I) at 33–35 weeks of gestation and 89 pregnant women delivering at term (38 weeks of gestation) (Group II). Enrollment was limited to primigravidae carrying singleton pregnancies. The exclusion was applied to any woman who reported smoking; recent use of antibiotics, antiseptics, or probiotic use; or a prior preterm birth, miscarriage, or multiple pregnancy. Recruitment was consecutive, covering every eligible woman during the study period, and no a priori sample size calculation was undertaken. The mean participant age was 26.2±1.9 years.

A diagnosis of preterm labor required at least two uterine contractions over a 10-minute interval together with cervical change at 33 to 35 weeks of gestation.

Controls were healthy pregnant women without obstetric, medical, or surgical complications, congenital anomalies, or histological evidence of chorioamnionitis. Routine postpartum histology of the placenta and fetal membranes was undertaken; since no case of histological chorioamnionitis was observed among controls, no woman was excluded for this reason.

All participants underwent a clinical examination, ultrasound evaluation, and microbiological testing.

Sample collection

Immediately before delivery, vaginal discharge was sampled from the mid-vaginal wall with sterile swabs. The smears were spread on glass slides, fixed in 90% ethanol and stained by the Gram method, 1% aqueous methylene blue, and Romanowsky staining.

Amniotic fluid was secured aseptically from the 120 women in the preterm labor group. For the 118 (98.3%) delivered by caesarean section, fluid was drawn directly from the amniotic cavity with a sterile needle once the operative field had been disinfected, immediately after the uterine incision and before the fetal membranes ruptured. In the 2 women (1.7%) who delivered vaginally after membrane rupture, a sterile catheter was used through the cervical canal under strict asepsis was used; neither of these two specimens produced microbial growth.

To limit possible contamination, the initial aliquot of aspirate was discarded; the remaining fluid was placed in sterile transport tubes and transferred to the microbiology laboratory in 2 h for analysis.

Microbiological analysis

Culture-based microbiology was performed on a range of selective and differential media: blood agar, egg yolk salt agar, Endo agar, *Lactobacillus*-selective agar, Sabouraud agar, Chrom *Candida* chromogenic medium, and selective agar for *Gardnerella vaginalis*.

Organisms were identified by their morphological, cultural, and biochemical properties following Bergey's Manual of Determinative Bacteriology, with automated confirmation on the Vitek 2 Compact system (bioMérieux, France). Specimens from both groups were processed under identical standard operating procedures, and microbiologists were blinded to the allocation of the clinical group during the culturing and identification.

The detection of *Ureaplasma* spp. was based on the Mycoplasma IST 2 system (bioMérieux, France), which allows both qualitative and quantitative readout. A count of $\geq 10^4$ colony-forming units (CFU) per sample was taken as clinically significant, and microbial load was reported as $\log_{10}$ CFU/mL.

Bacterial vaginosis was established on the Amsel criteria, at least three homogeneous vaginal discharges, vaginal pH > 4.5, clue cells, and a positive amine test, corroborated by bacterioscopy of stained smears. Vaginal pH was read with indicator strips.

Bacterioscopic assessment

Stained smears were examined under ×40 and ×100 objectives, recording leukocytes, epithelial cells, *Lactobacillus* spp., Gram-positive cocci, Gram-negative bacilli, yeast-like fungi, and clue cells.

Statistical analysis

Data were analyzed in IBM SPSS Statistics version 29 (Armonk, NY, USA). The Shapiro–Wilk test checked normality. Continuous variables appear as mean±standard deviation (SD); normally distributed data were compared by Student's or Welch's t test and skewed data by the nonparametric Mann–Whitney U test, as appropriate. Counts and percentages summarized categorical data, which were compared with Pearson's χ^2 or Fisher's exact test. Odds ratios (OR) and their 95% confidence intervals (CI) were derived for the link between microbial detection and preterm labor, with a continuity correction where a cell was zero. The φ-coefficient gauged the concordance between the detection of vaginal and amniotic fluid of *Ureaplasma* spp. within the preterm labor group.

Because every eligible participant in the study window was enrolled, no a priori sample size calculation was carried out. A post hoc power analysis, based on the observed difference in *Ureaplasma* spp. detection at $\alpha=0.05$, confirmed power above 80%. Due to the number of comparisons, p-values were left unadjusted for multiple testing and are treated as exploratory. Significance was set at $p<0.05$, with exact p-values given except where $p<0.001$. The analyzes were univariate: Given the exploratory, single-centre design, the fixed

enrolled sample, and the large number of microbial comparisons, no multivariate regression model adjusting for potential confounders, and associations are therefore reported unadjusted.

Ethical approval

The study adhered to the Declaration of Helsinki and was approved by the Bioethic Committee of the State Institution 'Center of Maternity and Childhood of the Ukrainian National Academy of Medical Sciences of Ukraine' (Protocol No. 3, April 20, 2023). Each participant provided written informed consent in writing before entering the study and a completed STROBE checklist is included with the manuscript as supplementary material.

Results

The detection frequencies of opportunistic organisms in vaginal samples from the preterm labor and term delivery groups are given in Table 1.

Table 1. Frequency of detection of opportunistic microorganisms in vaginal samples, n (%)*

Microorganism	**Preterm labour (n=120)**	**Term delivery (n=89)**	**OR**	**95% CI**	**p**
Lactobacillus spp.	86 (71.7)	89 (100)	0.01#	0.00–0.23	<0.001
Ureaplasma spp.	55 (45.8)	7 (7.9)	9.91	4.23–23.22	<0.001
Gardnerella vaginalis	73 (60.8)	10 (11.2)	12.27	5.78–26.06	<0.001
Candida spp.	25 (20.8)	10 (11.2)	2.08	0.94–4.59	0.07
Corynebacterium spp.	12 (10.0)	7 (7.9)	1.30	0.49–3.45	0.59
Klebsiella spp.	8 (6.7)	3 (3.4)	2.05	0.53–7.95	0.30
Escherichia coli	22 (18.3)	12 (13.5)	1.44	0.67–3.09	0.35
Haemolytic *E. coli*	16 (13.3)	0	28.26#	1.67–477.82	0.02
Enterococcus faecalis	22 (18.3)	11 (12.4)	1.59	0.73–3.48	0.24
Streptococcus pyogenes	12 (10.0)	0	20.62#	1.20–353.17	0.03
Streptococcus agalactiae	16 (13.3)	6 (6.7)	2.13	0.80–5.68	0.13
Staphylococcus aureus	8 (6.7)	0	13.52#	0.77–237.51	0.07
Staphylococcus epidermidis	32 (26.7)	10 (11.2)	2.87	1.33–6.22	0.006
Haemolytic *S. epidermidis*	20 (16.7)	3 (3.4)	5.73	1.65–19.96	0.006

* # – Odds ratio computed with a continuity correction for zero cells in the contingency table. For organisms found only in the preterm labor group, these estimates are imprecise and should be read with caution.

A reduced prevalence of *Lactobacillus* spp. was evident among women in preterm labor compared with term deliveries. In contrast, *Gardnerella vaginalis* and *Ureaplasma* spp. were recovered more frequently in this group. Within it, 53 cases (44.2%) showed *Ureaplasma* spp. at $\geq 10^4$ CFU/sample, whereas every term delivery stayed below that threshold.

Several additional opportunistic organisms were also more common in the preterm labor group, among them haemolytic *E. coli*, *S. pyogenes*, *S. aureus* and *S. epidermidis*.

Quantitatively, vaginal Gram-positive cocci reached higher loads in the preterm labor group, spanning $\log_{10}$ 4.0 to $\log_{10}$ 5.2 CFU/mL, against $\log_{10}$ 3.0 to $\log_{10}$ 3.8 CFU/mL at term ($p<0.001$).

Enterobacteriaceae were also more prevalent with preterm labor. Recovery of *E. coli*, haemolytic *E. coli*, and *Klebsiella* spp. was greater in this group, with loads of $\log_{10}$ 4.3 to $\log_{10}$ 5.4 CFU/mL versus $\log_{10}$ 3.2 to $\log_{10}$ 3.6 CFU/mL at term ($p<0.001$) (Fig. 1).

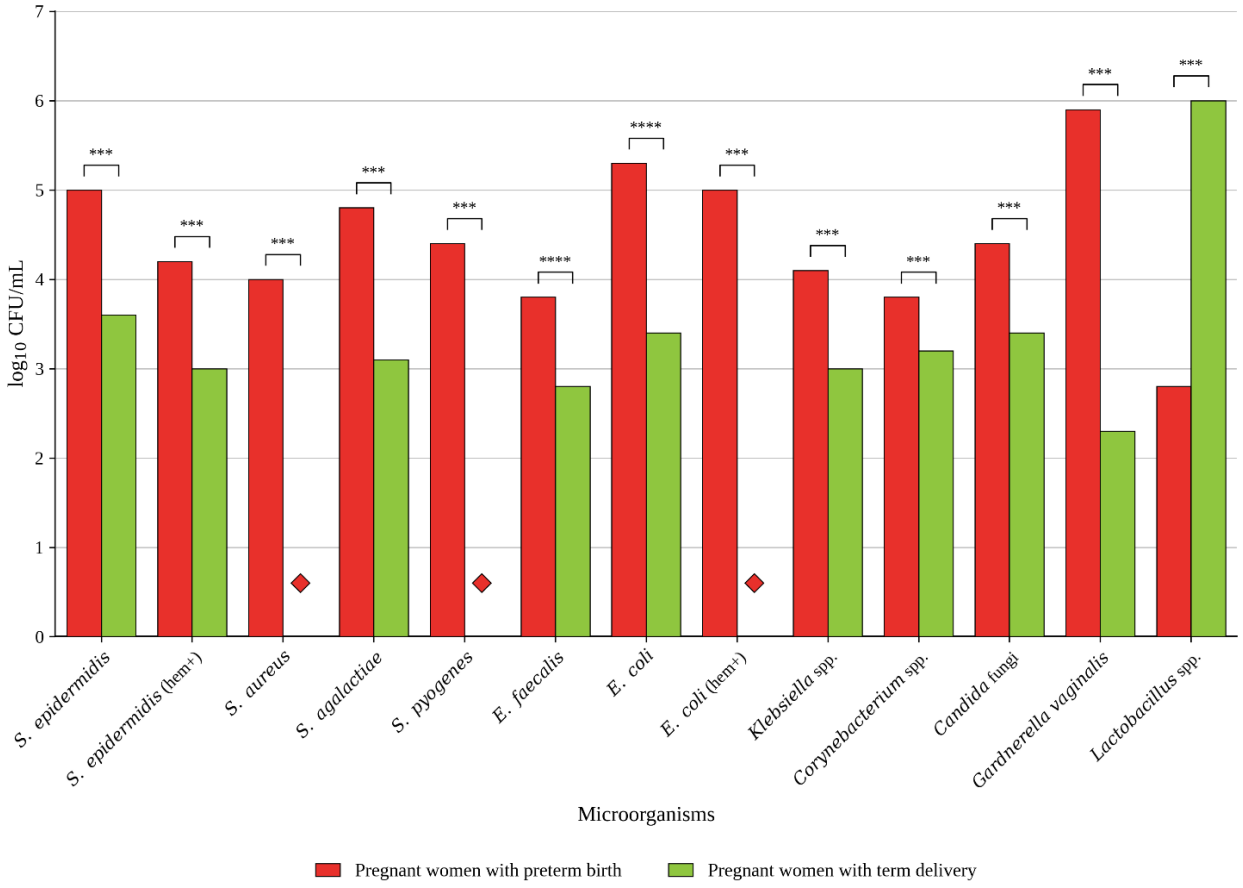

Fig. 1. Vaginal microbial loads in the preterm labor (n=120) and term delivery (n=89) groups, expressed as $\log_{10}$ CFU/mL and compared by the Mann–Whitney U test; *** $p<0.001$; **** $p<0.0001$; ♦ organism absent from the term delivery group

Candida spp. frequency was higher with preterm labour than at term. Among *Candida* isolates from the preterm labor group, *C. albicans* was dominant (21 cases, 17.5%), while nonalbicans species occurred less often and comprised *C. glabrata* (3 cases, 2.5%) and *C. tropicalis* (1 case, 0.8%). At term, only *C. albicans* was detected.

Vaginal pH exceeded 4.5 in 101 women (84.2%) of the preterm labor group, whereas at term it fell within 3.8 to 4.5.

Polymicrobial associations arose far more often with preterm labor than at term (61.7% vs. 13.5%). Frequent combinations were *S. epidermidis* with *Candida* spp., hemolytic *S. epidermidis* with *E. coli*, *E. coli* with *S. agalactiae* and *Candida* spp., and *Ureaplasma urealyticum* with *G. vaginalis*.

Bacterioscopy provided a further view of the vaginal microbiota. Smear-type distribution diverged between the preterm labor (n=120) and term (n=89) groups ($p<0.001$). At term, a normal pattern (normocenosis) prevailed in 86 women (96.6%). In the preterm labor group, by contrast, bacterial vaginosis was the leading finding (72 cases, 60.0%), followed by nonspecific vulvovaginitis (28 cases, 23.3%) and an intermediate type of smear in 20 women (16.7%).

Nonspecific vulvovaginitis featured elevated leukocyte counts (30-100 per field), mostly neutrophils, together with a greater number of Gram-positive cocci, Gram-negative bacilli, and *Candida* spp., in addition to a reduced presence of organisms resembling *Lactobacillus* spp. (Fig. 2).

Where there was bacterial vaginosis, the bacterioscopy showed a predominance of anaerobic morphotypes with moderate leukocyte response (15–30 per field); clue cells were common, as were organisms similar to *Mobiluncus* spp., *Fusobacterium* spp., and *Gardnerella* spp.

At term, bacterioscopy usually revealed *a Lactobacillus dominated* flora with few leukocytes (up to 10–12 per preparation) and few squamous epithelial cells (5–10 per preparation). Only three women (3.4%) here showed an intermediate smear type (Fig. 2).

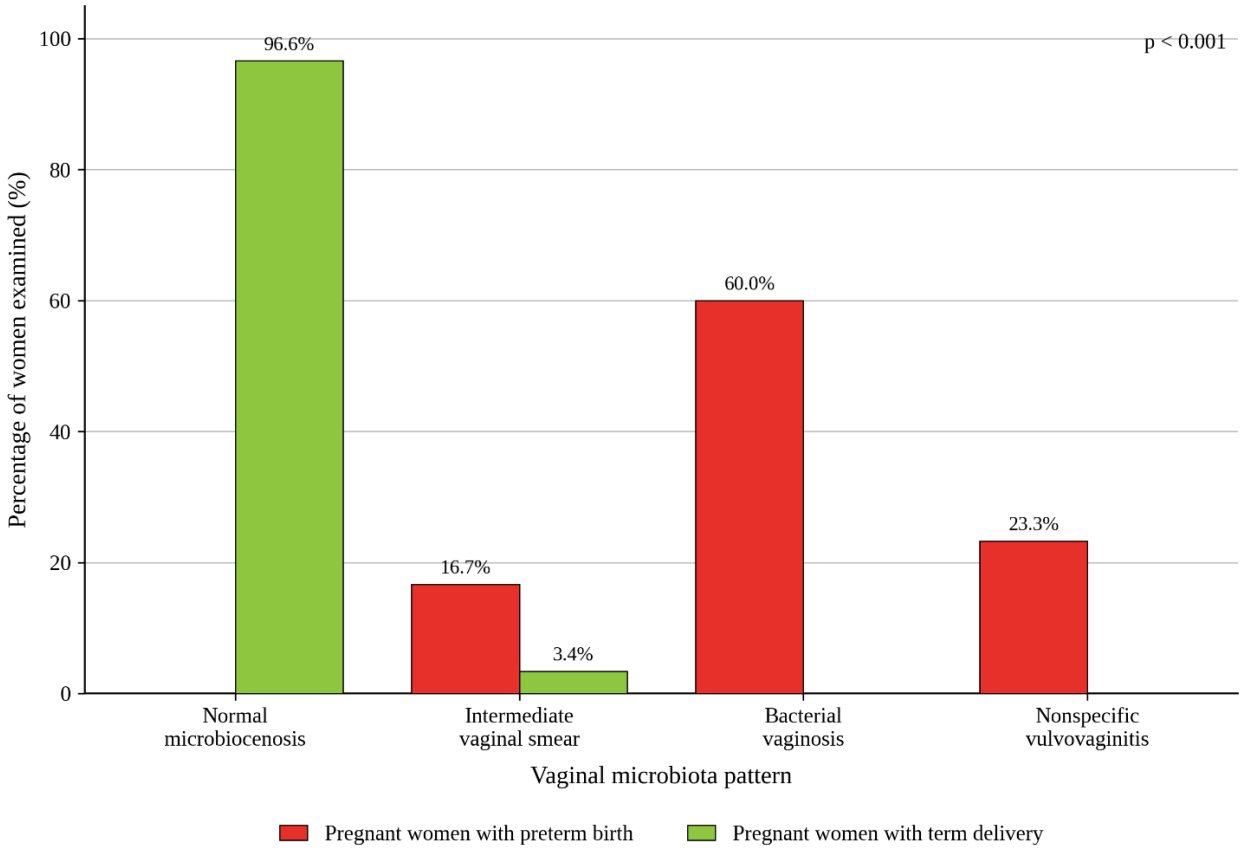

Fig. 2. Principal vaginal microbiota patterns in the preterm labor group and term delivery group; Values are percentages of the women examined.

A further aim was to determine the presence and makeup of organisms in amniotic fluid, sampled in all 120 women of the preterm labor group.

The amniotic fluid of the preterm labor group contained several Enterobacteriaceae. *E. coli* occurred in 14 women (11.7%), hemolytic *E. coli* in 9 cases (7.5%), and *E. faecalis* in 13 women (10.8%). Quantitative levels reached $\log_{10}$ 4.2 CFU/mL and $\log_{10}$ 4.4 CFU/mL, respectively. *Corynebacterium* spp. appeared in 5 cases (4.2%) at a lower load ($\log_{10}$ 2.2 CFU/mL). None of these were found at term.

Gram-positive cocci were also present in preterm amniotic fluid. *S. epidermidis* was identified in 19 women (15.8%), hemolytic *S. epidermidis* in 14 cases (11.7%), *S. aureus* in 5 cases (4.2%) and *S. agalactiae* in 11 cases (9.2%). Quantitative levels reached $\log_{10}$ 4.4 CFU/mL for *S. epidermidis* and $\log_{10}$ 4.2 CFU/mL for *S. aureus*.

Fungi of the genus *Candida* were recovered from amniotic fluid in 9 women (7.5%), though at loads under $\log_{10}$ 4.0 CFU/mL. *Lactobacillus* spp. were found in 14 cases (11.7%) at $\log_{10}$ 4.2 CFU/mL. *G. vaginalis* occurred in 5 women (4.2%) at $\log_{10}$ 3.8 CFU/mL.

Ureaplasma spp. were present in 29 cases (24.2%); in each, *Ureaplasma* spp. were also present vaginally. Loads reached $\geq 10^4$ CFU/sample in 18 women (15.0%), while 11 cases (9.2%) were below this cut-off

point. A positive association linked *Ureaplasma* spp. in vaginal and amniotic fluid specimens (φ=0.61; p<0.001).

Amniotic fluid remained sterile in 56 women (46.7%) of the preterm labor group; the other 64 (53.3%) grew organisms as single isolates or polymicrobial mixtures. The most common were *Ureaplasma* spp., *E. coli*, *E. faecalis*, *S. epidermidis*, *S. agalactiae*, and *C. albicans*. At term, the amniotic fluid was sterile in 82 women (92.1%), while 7 (7.9%) showed little growth of *S. epidermidis* at $\log_{10}$ 2.0 CFU/mL.

Comparing vaginal and amniotic fluid isolates within the preterm labor group revealed partial compositional concordance in 63 cases (52.5%) (Figure 3). In summary, three main findings emerged: preterm labor was marked by vaginal dysbiosis, with depleted *Lactobacillus* spp. along with *Gardnerella vaginalis* and *Ureaplasma* spp.; amniotic fluid produced microbial growth in 53.3% of preterm cases, while remaining sterile in the vast majority of term pregnancies; and vaginal and amniotic isolates showed partial concordance, a pattern compatible with ascending colonization.

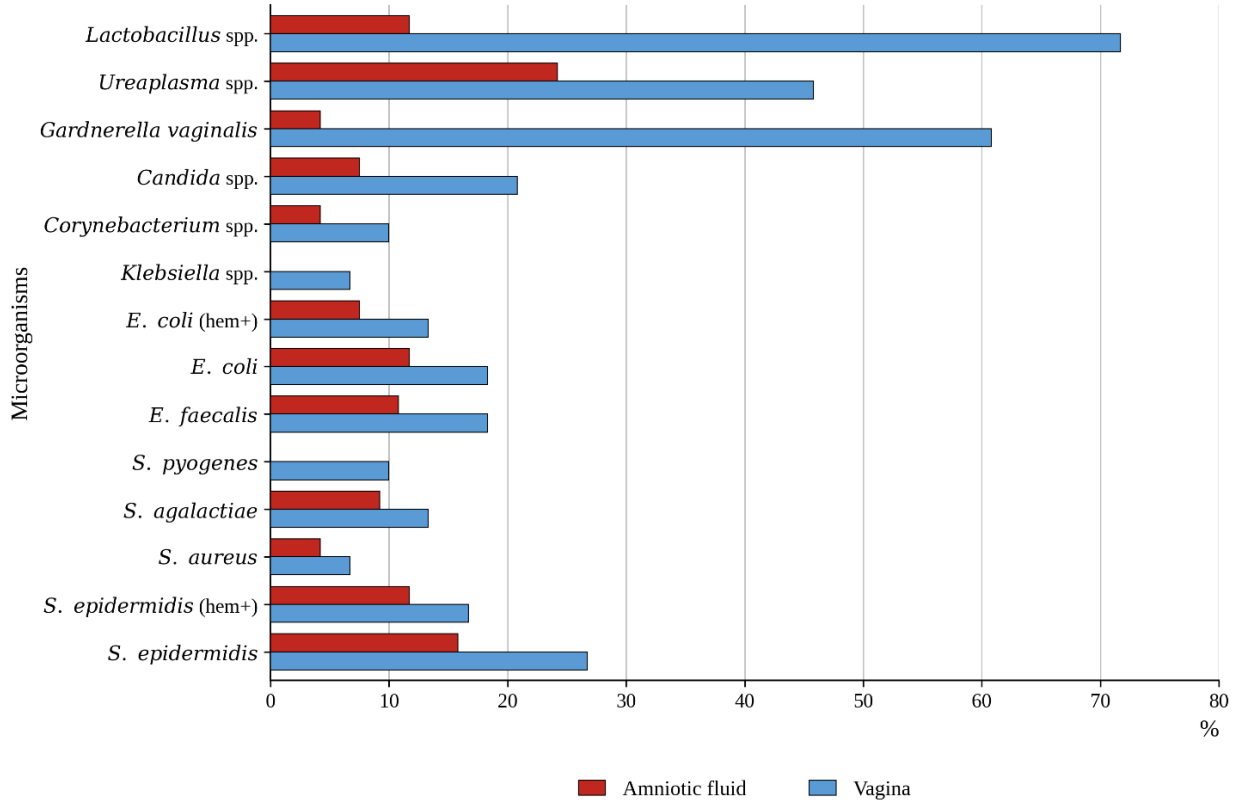

Fig. 3. Range of organisms recovered from vaginal and amniotic fluid specimens in the preterm labor group (n=120); values are percentages of women examined

Discussion

This study examined, by culture-based microbiology, the vaginal and amniotic fluid microbiota accompanying preterm labor. The results point to compositional differences between the preterm labor and term delivery groups.

The preterm labor group showed a lower prevalence of *Lactobacillus* spp. and a correspondingly greater recovery of opportunistic organisms such as *Staphylococcus* spp., Enterobacteriaceae, *Gardnerella vaginalis*, and *Ureaplasma* spp. This is consistent with previous reports that disruption of the vaginal microbial ecosystem often leads to preterm labour.[13,25]

Numerous large observational and molecular studies have described vaginal dysbiosis - a loss of dominance by *Lactobacillus* spp. with expansion of anaerobic and opportunistic organisms — as a correlate of adverse pregnancy outcomes.[25-27] Our culture-based data, showing heavier Gram-positive coccal loads in the preterm labor group, align with these reports.

In the present study, *Gardnerella vaginalis* emerged as one of the strongest correlates of preterm labor here, echoing earlier work on the role played by *G. vaginalis* in bacterial vaginosis, biofilm formation, and remodeling of the vaginal microbial environment.[19,28] *Ureaplasma* spp. were also recovered often and positively associated with preterm labour in our cohort, in keeping with reports connecting vaginal *Ureaplasma urealyticum* and *Mycoplasma hominis* with preterm delivery.[29,30]

A vaginal pH above 4.5 was seen more often with preterm labor. Previous work indicates that increasing pH can signal lactobacillus-dominated flora breakdown and overgrowth of opportunistic organisms.[31,32] Consistent with this, the term group held a physiological pH of 3.8 to 4.5.

Polymicrobial associations were also more frequent with preterm labor, possibly mirroring intricate interspecies interactions within the vaginal ecosystem.[33]

The amniotic fluid harbored organisms more often in the preterm labor group than at term, and vaginal and amniotic microbiota showed partial concordance in a percentage of patients. Taken together with the positive association for *Ureaplasma* spp., this may fit previously proposed ascending migration of organisms from the lower genital tract.[4,34,35] However, given the observational design and the possibility of sampling-related contamination, these results warrant caution and do not prove a causal ascending route.

A notable feature of this work is its reliance on culture. Although much current vaginal microbiome research is molecular, culture retains clinical utility by detecting viable organisms and quantifying load; molecular assays capture a wider set of taxa, including non-culturable ones, yet cannot confirm viability, so the two are best seen as complementary. Recovery of *Lactobacillus* spp. in every term of the participant exceeds the usual figures for molecular studies, most plausibly because culture favors dominant viable populations and because the control group was carefully selected on clinical grounds.

From a clinical perspective, the microbial patterns observed here may be of interest for future research. Because samples were obtained close to delivery, the present study does not assess the predictive value of

the vaginal microbiota or the efficacy of any intervention; The findings are hypothesis-generating and may motivate prospective studies by sampling earlier in pregnancy and incorporating inflammatory markers and molecular profiling – to explore whether such patterns could inform screening or preventive strategies. In routine obstetric practice, quantitative culture-based assessment could complement molecular microbiome testing, adding information on viable organisms and microbial load that sequencing alone does not capture, thus supporting future diagnostic or screening approaches in women at risk of preterm labor.

The strengths of the study include its relatively large cohort (n=209), quantitative culture-based analysis, and paired evaluation of vaginal and amniotic fluids.

Study limitations

Several caveats apply. First, the design was observational and cross-sectional, so cause and effect between microbial colonization and preterm labor cannot be drawn. Second, the sample size was not fixed a priori, given the design; a post hoc check, however, showed adequate power (>80%) for the main comparison. Third, culture has limited sensitivity, misses nonculturable or fastidious taxa, and may understate diversity relative to molecular sequencing. Fastidious anaerobes such as *Prevotella*, *Bacteroides*, *Atopobium*, *Mobiluncus*, *Peptostreptococcus*, and *Fusobacterium* spp. went undetected by culture; their absence reflects methodological limits rather than true absence, and bacterioscopy did show matching anaerobic morphotypes where bacterial vaginosis was present. Fourth, no multivariable adjustment was made, so residual confounding remains possible; factors such as antibiotic exposure, timing of membrane rupture, prior genital infection, and smoking were not modelled, and baseline variables were not uniformly captured, so the associations are unadjusted. Fifth, though amniotic fluid was taken aseptically, contamination cannot be wholly excluded, especially after membrane rupture, which bears on the apparent vaginal–amniotic similarity. Sixth, restriction to 33–35 weeks of gestation may curb generalizability to other gestational ages. Finally, inflammatory markers such as interleukin-6 and C-reactive protein were not assayed, so microbial colonization could not be distinguished from an intra-amniotic inflammatory response.

Conclusion

Vaginal dysbiosis – marked by fewer *Lactobacillus* spp., more frequent *G. vaginalis* and *Ureaplasma* spp., raised vaginal pH, and abundant polymicrobial associations – was seen more often with preterm labor than at term.

Amniotic fluid yielded organisms more often in the preterm labor group, with partial vaginal–amniotic concordance in a subset of cases. These observations are compatible with, but do not establish, the previously proposed ascending migration of organisms from the lower genital tract.

Quantitative culture-based evaluation of the vaginal microbiota may add microbiological detail useful for appraising colonization patterns in pregnancy, bearing in mind the limited sensitivity of culture. Prospective

longitudinal studies combining culture-based and molecular microbiome analyses are warranted to validate the clinical applicability of these findings.

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Declarations

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Author contributions

Conceptualization, I.P. and T.L.; Methodology, T.L.; Software, M.L.; Validation, I.P.; Formal Analysis, T.L.; Investigation, T.L. and M.L.; Resources, T.L.; Data Curation, I.P.; Writing – Original Draft Preparation, T.L.; Writing – Review & Editing, I.P. and M.L.; Visualization, M.L. and T.L.; Supervision, I.P.; Project Administration, I.P.

Conflicts of interest

The authors report no competing interests.

Data availability

Datasets produced or analyzed in this study can be obtained from the corresponding author on reasonable request.

Ethics approval

The study followed the Declaration of Helsinki and received approval from the Bioethics Committee of the State Institution "Ukrainian Center of Maternity and Childhood of the National Academy of Medical Sciences of Ukraine" (Protocol No. 3, April 20, 2023).

Use of AI and AI-assisted technologies in the writing process

In preparing this work, the authors employed AI-assisted language-editing tools to enhance clarity and readability. Having used these tools, the authors then reviewed and revised the text as required and accept full responsibility for the content of the published article.

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