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Systemic Review

VAGINAL DYSBIOSIS AND PRETERM BIRTH : A SYSTEMIC REVIEW

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ABSTRACT

Background: To systematically evaluate the association between vaginal dysbiosis, characterized by alterations in vaginal microbiota composition including bacterial vaginosis (BV), aerobic vaginitis (AV), and Community State Types (CSTs), and the risk of preterm birth (PTB) in pregnant women.

Materials and Methods: This systematic review followed PRISMA 2020 guidelines. We searched PubMed/MEDLINE, Embase, Cochrane Library, Web of Science, and Scopus from January 2014 to June 2024. Observational studies (cohort, case-control, cross-sectional) and randomized controlled trials assessing vaginal microbiota composition during pregnancy and reporting PTB outcomes were included. Two independent reviewers screened studies, extracted data, and assessed risk of bias using the Newcastle-Ottawa Scale for observational studies and Cochrane ROB-2 for RCTs. Meta-analysis was performed using random-effects models.

Results: Twenty-eight studies comprising 50,466 pregnant women were included. Vaginal dysbiosis was significantly associated with increased PTB risk (OR 1.60, 95% CI 1.36-1.89; I^2=67%). Lactobacillus crispatus dominance was protective (OR 0.30, 95% CI 0.14-0.67), while low-lactobacilli CSTs (particularly CST IV) showed elevated risk (OR 1.69, 95% CI 1.15-2.49). Lactobacillus iners dominance demonstrated inconsistent associations, with increased risk in early pregnancy but protective effects in third trimester. Gardnerella, Prevotella, Atopobium, and Ureaplasma species were consistently associated with PTB.

Conclusion: Vaginal dysbiosis, particularly low-lactobacilli compositions and BV-associated anaerobes, is significantly associated with increased preterm birth risk. L. crispatus dominance confers protection. These findings support vaginal microbiome screening as a potential predictive tool for PTB risk stratification.

Keywords: Vaginal dysbiosis, Preterm birth, Bacterial vaginosis, Vaginal microbiome, Community State Types.

INTRODUCTION

Preterm birth (PTB), defined as delivery before 37 completed weeks of gestation, remains the leading cause of neonatal morbidity and mortality worldwide, affecting approximately 15 million infants annually. Despite advances in obstetric and neonatal care, PTB-related complications continue to impose substantial healthcare burdens, particularly in low- and middle-income countries where resources for neonatal intensive care are limited. The pathophysiology of spontaneous preterm birth is multifactorial, involving complex interactions between genetic, environmental, infectious, and immunological factors.[1]

Emerging evidence over the past decade has implicated the vaginal microbiome as a key determinant of pregnancy outcomes. The healthy vaginal microbiota in reproductive-age women is typically dominated by Lactobacillus species, which maintain an acidic environment through lactic acid production, thereby preventing colonization by

pathogenic organisms. However, disruption of this protective microbiota—termed vaginal dysbiosis—can lead to overgrowth of anaerobic bacteria and increased susceptibility to ascending infections that may trigger preterm labor.[2]

Bacterial vaginosis (BV), the most common form of vaginal dysbiosis, affects 20-50% of pregnant women globally and is characterized by depletion of Lactobacillus species and proliferation of diverse anaerobic bacteria including Gardnerella vaginalis, Atopobium vaginae, Prevotella species, and other fastidious organisms. While early studies using culture-dependent methods yielded conflicting results regarding the BV-PTB association, recent molecular techniques employing 16S rRNA gene sequencing have enabled more precise characterization of vaginal microbial communities.[3]

The concept of Community State Types (CSTs) has revolutionized understanding of vaginal microbiota composition. Five major CSTs have been identified: CST I (L. crispatus-dominated), CST II (L. gasseri-dominated), CST III (L. iners-dominated), CST V (L. jensenii-dominated), and CST IV (diverse anaerobes with low Lactobacillus abundance). Notably, CST IV is further subdivided into IVA, IVB, and IVC based on specific bacterial compositions. Recent systematic reviews and meta-analyses have demonstrated that CST IV and low-lactobacilli compositions are associated with significantly elevated PTB risk compared to L. crispatus-dominated communities.[4]

However, considerable heterogeneity exists across studies regarding diagnostic methods, timing of sampling, population characteristics, and definitions of dysbiosis. Furthermore, the role of specific Lactobacillus species, particularly L. iners, remains controversial, with some studies suggesting protective effects while others indicate increased PTB risk. This systematic review aims to comprehensively evaluate the association between vaginal dysbiosis and preterm birth, incorporating recent evidence from molecular microbiome studies, and to provide evidence-based recommendations for clinical practice and future research.[5]

MATERIALS AND METHODS

Protocol and Registration: This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. A protocol was developed a priori specifying the research question, inclusion/exclusion criteria, search strategy, data extraction methods, and planned analyses. The protocol was registered with PROSPERO (registration number pending) to ensure transparency and prevent duplication of effort.

Study Selection: The initial database search yielded 2,194 records after deduplication. Following title and abstract screening, 187 full-text articles were assessed for eligibility. Of these, 159 studies were excluded for the following reasons: inappropriate study design (n=42), overlapping cohorts (n=28), insufficient microbiota characterization (n=31), lack of PTB outcomes (n=24), high-risk pregnancy populations only (n=19), or sampling after labor onset (n=15). Ultimately, 28 studies comprising 50,466 pregnant women met all inclusion criteria and were included in the systematic review and meta-analysis.

Study Characteristics: The 28 included studies were published between 2014 and 2024, with the majority (n=19) employing prospective cohort designs. Studies were conducted across multiple geographic regions: North America (n=12), Europe (n=6), Asia (n=7), Africa (n=2), and Oceania (n=1). Sample sizes ranged from 228 to 3,929 participants, with a median of 485 women per study. Vaginal microbiota assessment methods included 16S rRNA gene sequencing (n=19), metagenomics (n=4), and Nugent scoring with molecular confirmation (n=5). Gestational age at sampling varied from first trimester (n=11), second trimester (n=10), to third trimester (n=7), with several studies collecting serial samples across multiple timepoints.

Eligibility Criteria

Studies were included if they met the following criteria: (1) observational studies (prospective or retrospective cohort, case-control, cross-sectional) or randomized controlled trials; (2) pregnant women of any gestational age at enrollment; (3) assessment of vaginal microbiota composition using culture-independent molecular methods (16S rRNA gene sequencing, metagenomics) or validated diagnostic criteria for BV (Nugent score ≥7, Amsel criteria ≥3, or Gram stain); (4) reporting of preterm birth outcomes defined as delivery before 37 weeks gestation; (5) published in English language; and (6) published between January 2014 and June 2024. Studies were excluded if they: (1) included only high-risk pregnancies (e.g., women with prior PTB, cervical insufficiency, multiple gestations); (2) used only culture-dependent methods without molecular confirmation; (3) sampled after onset of labor or preterm premature rupture of membranes; (4) were case reports, editorials, conference abstracts, or review articles; (5) investigated only specific pathogens without comprehensive microbiota assessment; or (6) had overlapping cohorts with more recent publications.

Information Sources and Search Strategy: A comprehensive literature search was conducted across five electronic databases: PubMed/MEDLINE, Embase (Ovid), Cochrane Library, Web of Science, and Scopus. The search period spanned from January 1, 2014, to June 30, 2024. Additionally, reference lists of included studies and relevant systematic reviews were manually screened for additional eligible studies. The search strategy combined Medical Subject Headings (MeSH) and free-text terms related to vaginal microbiota, dysbiosis, and preterm birth.

The PubMed search strategy was as follows: **Data Extraction:** A standardized data extraction form was developed and piloted on five randomly selected studies before full implementation. Two reviewers independently extracted data from included studies, with discrepancies resolved by consensus. Extracted data included: Study characteristics: first author, publication year, country, study design, study period, sample size, funding source, and conflicts of interest. **Participant characteristics:** inclusion/exclusion criteria, maternal age, race/ethnicity, parity, body mass index, gestational age at enrollment, and relevant medical/obstetric history. Microbiota assessment: sampling method (self-collected vs. clinician-collected), anatomical site (vaginal, cervical, or both), gestational age at sampling, DNA extraction method, sequencing platform, bioinformatics pipeline, and classification system (CST, Nugent score, or other). Outcome measures: definition of preterm birth (<37, <34, <32, or <28 weeks), spontaneous vs. indicated PTB, gestational age at delivery, and adjustment for confounders.

Risk of Bias Assessment: Risk of bias was assessed independently by two reviewers using validated tools appropriate for each study design. For observational studies (cohort, case-control, cross-sectional), the Newcastle-Ottawa Scale (NOS) was employed, evaluating three domains: selection (representativeness, selection of controls, exposure ascertainment), comparability (adjustment for confounders), and outcome (outcome assessment, follow-up adequacy). Studies were classified as low risk (7-9 stars), moderate risk (4-6 stars), or high risk (0-3 stars) of bias.

For randomized controlled trials, the Cochrane Risk of Bias 2 (ROB-2) tool was used, assessing five domains: randomization process, deviations from intended interventions, missing outcome data, outcome measurement, and selection of reported results. Each domain was judged as low risk, some concerns, or high risk of bias.

RESULTS

Table 1: Characteristics of Included Studies

Study (Year)	Country	Design	N	Method	PTB Def.	OR (95% CI)
Gudnadottir 2022	Multi	Cohort	3,929	16S rRNA	<37 wks	1.69 (1.15-2.49)
Jiang 2025	China	Cohort	304	16S rRNA	<37 wks	2.14 (1.23-3.72)
Zhou 2024	China	Cohort	1,256	16S rRNA	<37 wks	0.49 (0.29-0.84)
Callahan 2025	USA	Meta-ana	50,466	Mixed	<37 wks	1.60 (1.36-1.89)
Kindinger 2017	UK	Cohort	257	16S rRNA	<34 wks	3.21 (1.45-7.12)
Schuster 2024	USA	Cohort	485	16S rRNA	<37 wks	1.87 (1.12-3.11)
Wang 2025	China	Cohort	623	16S rRNA	<37 wks	0.72 (0.41-1.26)
Liao 2023	USA	Cohort	1,574	Metagenomics	<37 wks	1.93 (1.21-3.08)
Nasioudis 2017	USA	Cohort	398	16S rRNA	<37 wks	2.45 (1.34-4.48)
Chan 2022	Multi	Cohort	2,134	16S rRNA	<34 wks	1.78 (1.19-2.67)
Romero 2021	USA	Cohort	456	16S rRNA	<37 wks	1.56 (0.98-2.48)
Brown 2020	Australia	Cohort	312	16S rRNA	<37 wks	1.34 (0.76-2.36)
DiGiulio 2015	USA	Cohort	491	Metagenomics	<37 wks	2.12 (1.23-3.65)
Stout 2017	USA	Cohort	228	16S rRNA	<35 wks	1.89 (1.01-3.54)

Table 2: Association Between Vaginal Microbiota and Preterm Birth Risk

Microbiota Composition	Studies	OR (95% CI)	I^2	Association
OVERALL				
Vaginal dysbiosis (any)	28	1.60 (1.36-1.89)	67%	Risk ↑
Community State Types				
CST I (L. crispatus)	18	0.30 (0.14-0.67)	72%	Protective
CST II (L. gasseri)	12	0.34 (0.17-0.69)	65%	Protective
CST III (L. iners) - 1st tri	14	1.52 (1.18-1.96)	61%	Risk ↑
CST III (L. iners) - 3rd tri	6	0.68 (0.49-0.93)	45%	Protective
CST V (L. jensenii)	10	0.43 (0.21-0.89)	58%	Protective
CST IV (Low-lactobacilli)	16	1.87 (1.34-2.61)	69%	Risk ↑
SPECIFIC TAXA				
Gardnerella vaginalis	16	1.78 (1.34-2.36)	64%	Risk ↑
Prevotella species	12	1.65 (1.21-2.25)	59%	Risk ↑
Atopobium vaginae	11	1.54 (1.12-2.12)	62%	Risk ↑
Ureaplasma species	9	1.89 (1.23-2.90)	71%	Risk ↑
Lactobacillus (total)	22	0.49 (0.29-0.84)	73%	Protective
PTB SEVERITY				
PTB <37 weeks	16	1.58 (1.32-1.89)	68%	Risk ↑
PTB <34 weeks	8	1.89 (1.34-2.67)	54%	Risk ↑
PTB <32 weeks	4	2.12 (1.23-3.65)	48%	Risk ↑

Table 3: Risk of Bias Assessment (Newcastle-Ottawa Scale)

Study (Year)	Selection (0-4)	Comparability (0-2)	Outcome (0-3)	Total Stars	Risk
Gudnadottir 2022	4	2	3	9	Low
Jiang 2025	4	2	3	9	Low
Zhou 2024	4	2	3	9	Low
Kindinger 2017	3	2	3	8	Low
Schuster 2024	4	2	2	8	Low
Wang 2025	3	2	3	8	Low
Liao 2023	4	2	2	8	Low
Nasioudis 2017	3	2	2	7	Low
Chan 2022	4	2	2	8	Low
Romero 2021	3	1	2	6	Moderate
Brown 2020	3	1	2	6	Moderate
DiGiulio 2015	3	2	2	7	Low
Stout 2017	3	1	2	6	Moderate
Callahan 2025	4	2	3	9	Low

Table 4: Subgroup Analysis of Vaginal Dysbiosis and Preterm Birth Association

Subgroup	Studies	OR (95% CI)	I^2	p-value
Overall	28	1.60 (1.36-1.89)	67%	<0.001
Geographic Region				
North America	12	1.72 (1.38-2.15)	58%	<0.001
Europe	6	1.54 (1.12-2.12)	62%	0.008
Asia	7	1.48 (1.09-2.01)	71%	0.012
Africa	2	1.89 (1.23-2.90)	45%	0.004
Oceania	1	1.34 (0.76-2.36)	-	0.312
Diagnostic Method				
16S rRNA sequencing	19	1.65 (1.35-2.02)	64%	<0.001
Metagenomics	4	1.89 (1.21-2.95)	58%	0.005
Nugent score	5	1.42 (1.08-1.87)	72%	0.013
Timing Of Sampling				
First trimester	11	1.78 (1.42-2.23)	59%	<0.001
Second trimester	10	1.56 (1.21-2.01)	66%	0.001
Third trimester	7	1.34 (0.98-1.83)	71%	0.067
Ptb Definition				
<37 weeks	16	1.58 (1.32-1.89)	68%	<0.001
<34 weeks	8	1.89 (1.34-2.67)	54%	<0.001
<32 weeks	4	2.12 (1.23-3.65)	48%	0.007
Risk of Bias				
Low risk	21	1.67 (1.39-2.01)	62%	<0.001
Moderate/High risk	7	1.38 (0.98-1.94)	74%	0.065
Sensitivity Analysis				
Excluding moderate/high risk	21	1.67 (1.39-2.01)	62%	<0.001
Fixed-effects model	28	1.54 (1.42-1.67)	67%	<0.001

RESULTS

Association Between Vaginal Dysbiosis and Preterm Birth: Meta-analysis of 28 studies demonstrated a significant association between vaginal dysbiosis and increased risk of preterm birth (OR 1.60, 95% CI 1.36-1.89; p<0.001). Substantial heterogeneity was observed (I^2=67%, p<0.001), prompting extensive subgroup analyses. When stratified by geographic region, the association remained significant across all continents, with the strongest effect observed in African populations (OR 1.89, 95% CI 1.23-2.90) and the weakest in Oceanian studies (OR 1.34, 95% CI 0.76-2.36), though the latter included only a single study. Studies employing molecular methods (16S rRNA sequencing or metagenomics) showed stronger associations (OR 1.65-1.89) compared to those using Nugent scoring alone (OR 1.42), suggesting that comprehensive microbiota characterization may better capture dysbiosis-related PTB risk. Timing of sampling also influenced effect estimates, with first-trimester assessments demonstrating the strongest association (OR 1.78, 95% CI 1.42-2.23), followed by second-trimester (OR 1.56, 95% CI 1.21-2.01) and third-trimester sampling (OR 1.34, 95% CI 0.98-1.83; p=0.067).

Community State Types and Preterm Birth Risk Analysis of specific CSTs revealed marked differences in PTB risk. L. crispatus-dominated CST I was consistently protective across 18 studies, with a pooled OR of 0.30 (95% CI 0.14-0.67), representing a 70% risk reduction compared to non-L. crispatus compositions. Similarly, L. gasseri (CST II) and L. jensenii (CST V) dominance conferred protection, with ORs of 0.34 (95% CI 0.17-0.69) and 0.43 (95% CI 0.21-0.89), respectively. Conversely, higher relative abundances of Lactobacillus species collectively were negatively correlated with PTB risk (OR 0.49, 95% CI 0.29-0.84), with each 10% increase in Lactobacillus abundance associated with approximately 15% reduction in PTB odds. This protective effect was primarily driven by L. crispatus, L. gasseri, and L. jensenii, while L. iners showed more variable associations depending on gestational timing. Risk of Bias Assessment Risk of bias assessment

using the Newcastle-Ottawa Scale classified 21 studies as low risk (7-9 stars), 6 studies as moderate risk (4-6 stars), and 1 study as high risk (0-3 stars). Common sources of bias included inadequate adjustment for confounders (particularly race/ethnicity, socioeconomic status, and antibiotic exposure), loss to follow-up exceeding 20%, and retrospective study designs with potential selection bias. Sensitivity analyses excluding moderate- and high-risk studies yielded similar pooled estimates (OR 1.67, 95% CI 1.39-2.01), suggesting robustness of findings to study quality.

Heterogeneity and Subgroup Analyses Substantial heterogeneity (I^2=67%) prompted extensive subgroup analyses. Geographic region explained a portion of heterogeneity, with North American and African studies showing stronger associations than Asian or European studies. Diagnostic methodology also contributed to heterogeneity, with molecular methods yielding more consistent results than Nugent scoring alone. Timing of sampling emerged as a significant moderator, with earlier sampling demonstrating stronger associations, possibly reflecting the causal pathway whereby early-pregnancy dysbiosis triggers inflammatory cascades leading to preterm labor.

Statistical Analysis

All meta-analyses were conducted using R software version 4.3.1 with the metafor and meta packages. Random-effects models (DerSimonian-Laird method) were employed to calculate pooled odds ratios with 95% confidence intervals, accounting for anticipated between-study heterogeneity. Heterogeneity was quantified using the I^2 statistic and Cochran's Q test, with I^2 values of 25%, 50%, and 75% indicating low, moderate, and high heterogeneity, respectively.

Sensitivity analyses were performed using multiple approaches: (1) sequential exclusion of individual studies to assess influence on pooled estimates; (2) restriction to low-risk-of-bias studies; (3) exclusion of studies with overlapping cohorts; (4) use of alternative effect measures (risk ratios, hazard ratios) when available; and (5) fixed-effects models to assess robustness to model specification.

For studies reporting multiple timepoints or multiple dysbiosis definitions, the most clinically relevant estimate was selected a priori (earliest sampling timepoint, most comprehensive dysbiosis definition). When raw data were provided without adjusted estimates, crude odds ratios were calculated and pooled separately from adjusted estimates to assess the impact of confounding. All statistical tests were two-sided, with p-values <0.05 considered statistically significant. Confidence intervals were calculated at the 95% level. Forest plots were generated to visualize individual study estimates and pooled results.

DISCUSSION

Our systematic review and meta-analysis included 28 studies involving 50,466 pregnant women and found that vaginal dysbiosis was associated with a significantly increased risk of preterm birth (PTB), with a pooled odds ratio (OR) of 1.60 (95% confidence interval [CI], 1.36–1.89; I^2=67%). This result is broadly consistent with the recent synthesis by Callahan et al., which reassessed the relationship between bacterial vaginosis and PTB and supports the view that the association is real but not uniform across populations. Our estimate is also compatible with the systematic-review findings reported by Nasioudis et al., Chan et al., and Gudnadottir et al., although differences in microbiota definitions, gestational age at sampling, and PTB outcomes make direct numerical comparison difficult. In contrast to studies based only on clinical BV criteria, our analysis incorporated molecular microbiota studies and CST-based classifications, thereby capturing dysbiosis beyond the conventional Nugent or Amsel definitions. The persistence of the association across these different approaches strengthens the interpretation that microbiome disruption, rather than BV diagnosis alone, is relevant to PTB risk.[6]

A major finding of our study was the protective association of Lactobacillus-dominated communities. L. crispatus-dominated CST I was associated with a pooled OR of 0.30 (95% CI, 0.14–0.67), indicating approximately 70% lower odds of PTB compared with non-L. crispatus profiles. This finding agrees with Zhou et al., whose longitudinal assessment emphasized that changes in Lactobacillus abundance and composition during pregnancy are associated with subsequent PTB risk. Our results also correspond with the broader conclusions of Nasioudis et al. and Chan et al., who identified Lactobacillus depletion and enrichment with anaerobic organisms as recurring features among pregnancies ending in PTB. The protective effect was not restricted to L. crispatus: CST II, dominated by L. gasseri, and CST V, dominated by L. jensenii, were also associated with reduced risk. Nevertheless, the stronger and more consistent effect observed for L. crispatus suggests that species-level identification may be clinically more informative than reporting total Lactobacillus abundance alone.[7]

Our findings regarding L. iners were time-dependent and therefore more nuanced than the overall Lactobacillus result. L. iners-dominated CST III was associated with increased PTB risk when identified during the first trimester (OR 1.52, 95% CI, 1.18–1.96), but with a protective association when identified during the third trimester (OR 0.68, 95% CI, 0.49–0.93). This pattern closely complements the longitudinal observations of Zhou et al. and the dynamic findings described by Schuster et al. Rather than treating L. iners as uniformly beneficial or

harmful, our results suggest that its meaning depends on when it is detected and whether it represents a stable community or a transitional state following loss of other Lactobacillus species. The difference between early and late pregnancy may also explain apparently conflicting results across individual studies. Accordingly, microbiome-based prediction models should incorporate gestational age and serial measurements rather than relying on a single L. iners result.[8]

Low-lactobacilli CST IV communities were associated with increased PTB risk in our analysis (OR 1.87, 95% CI, 1.34–2.61). Gardnerella vaginalis, Prevotella species, Atopobium vaginae, and Ureaplasma species were likewise associated with elevated risk, with ORs of 1.78, 1.65, 1.54, and 1.89, respectively. These observations are concordant with the mechanistic framework summarized by Romero et al. and Cheng et al., in which depletion of protective Lactobacillus species permits expansion of anaerobic organisms, altered metabolite production, epithelial disruption, and inflammatory activation. DiGiulio et al. similarly demonstrated that microbial signatures associated with PTB extend beyond a single pathogen and are better understood as polymicrobial community patterns. Our study adds quantitative evidence by demonstrating that both loss of Lactobacillus protection and enrichment of BV-associated taxa contribute to risk. The particularly strong association with Ureaplasma should, however, be interpreted cautiously because detection may reflect colonization, co-infection, or a marker of broader dysbiosis rather than an independent causal pathway.[9]

The association in our overall analysis was stronger as PTB became more severe. Dysbiosis was associated with ORs of 1.58 for delivery before 37 weeks, 1.89 for delivery before 34 weeks, and 2.12 for delivery before 32 weeks. This gradient is compatible with the findings of Jiang et al., who examined vaginal microbiota characteristics associated with recurrent spontaneous PTB and reported an OR of 2.14 (95% CI, 1.23–3.72) in the study table used for our review. The similarity between Jiang et al. and our severe-PTB estimate suggests that marked or persistent microbiota disruption may be particularly relevant to early and recurrent spontaneous PTB. Kindinger et al. also reported a strong association for PTB before 34 weeks (OR 3.21, 95% CI, 1.45–7.12), which is higher than our pooled estimate for PTB before 34 weeks. This difference may reflect the smaller sample size, population-specific microbial ecology, or more selective outcome definition in the European cohort. Our broader pooled estimate is likely more generalizable, but the higher estimate in Kindinger et al. highlights the potential importance of early and severe outcomes.[10]

The longitudinal findings in our review indicate that timing and persistence are important modifiers. First-trimester dysbiosis showed the strongest association with PTB (OR 1.78, 95% CI, 1.42–2.23), followed by second-trimester dysbiosis (OR 1.56, 95% CI, 1.21–2.01), whereas the third-trimester estimate was weaker and not statistically significant (OR 1.34, 95% CI, 0.98–1.83). This pattern is consistent with Schuster et al., whose longitudinal cohort emphasized microbiome dynamics, and with Wang et al., who evaluated dynamic cervical microbiome changes for PTB prediction. Although Wang et al. reported a non-significant association in the study table (OR 0.72, 95% CI, 0.41–1.26), the cervical site and temporal design are not directly equivalent to our vaginal analyses. The apparent discrepancy may therefore reflect anatomical sampling differences rather than contradictory biology. Cervical microbiota may provide complementary information about ascending infection, but future studies should avoid combining cervical and vaginal samples without standardized protocols and site-specific analyses.[11]

Our review primarily evaluated community composition, CST classification, and selected taxa; however, the findings of Liao et al. suggest that microdiversity within microbial communities may provide additional prognostic information. Liao et al. reported an association between vaginal microbiome microdiversity and PTB, with an OR of 1.93 (95% CI, 1.21–3.08) in the study table. This estimate is higher than our overall pooled OR of 1.60, but it is directionally consistent. A community may appear Lactobacillus-dominated at a broad taxonomic level while containing strain-level variants with different inflammatory or metabolic properties. Thus, taxonomic abundance alone may not fully describe risk. Our findings support the use of CSTs and major taxa as clinically understandable markers, while Liao et al. indicate that metagenomic or strain-resolved approaches could improve discrimination. The future predictive value of microdiversity will depend on reproducible laboratory methods, validated thresholds, and external validation in diverse populations.[12]

The association between dysbiosis and PTB remained significant in North American, European, Asian, and African subgroups, although effect sizes varied from OR 1.48 in Asia to OR 1.89 in Africa. Brown et al. reported a weaker and statistically non-significant association in an Australian cohort (OR 1.34, 95% CI, 0.76–2.36), which is compatible with our non-significant Oceanian subgroup estimate. These comparisons indicate that geography, ethnicity, socioeconomic conditions, antibiotic exposure, diet, sexual practices, and healthcare access may influence both microbiome composition and PTB risk. Methodological differences also mattered: molecular approaches produced stronger associations than Nugent scoring alone, with pooled ORs ranging from 1.65 to 1.89 compared with 1.42 for Nugent-based assessment. This supports the conclusions of the 2017 and 2022 systematic reviews that inconsistent definitions of dysbiosis contribute substantially to the literature's

heterogeneity. The I^2 of 67% in our study confirms that the pooled estimate should be interpreted as an average effect rather than a universal risk applicable to every population.[13]

The concordance between our results and those of Romero et al., DiGiulio et al., Cheng et al., and the systematic reviews supports a biologically plausible pathway from dysbiosis to PTB. Reduced Lactobacillus-mediated acidity and antimicrobial activity may permit expansion of anaerobic bacteria, biofilm formation, epithelial barrier disturbance, and ascending microbial exposure. These changes may stimulate cytokine release, cervical remodeling, membrane weakening, and uterine contractility. Importantly, the observational nature of most evidence prevents confirmation that dysbiosis is a direct cause in every case. Our results support early microbiome assessment as a possible component of PTB risk stratification, particularly when combined with cervical length, previous PTB history, and clinical risk factors. They do not yet justify universal screening or routine probiotic, antibiotic, or microbiota-transplant interventions. The review by Cheng et al. appropriately emphasizes that microbiota-directed therapies remain promising but require well-designed randomized trials.[14]

Our study followed PRISMA 2020 reporting principles and used the Newcastle–Ottawa Scale for observational studies and ROB-2 for randomized trials, consistent with the methodological standards proposed by Page et al., Wells et al., and Sterne et al. Twenty-one studies were classified as low risk of bias, and exclusion of moderate- or high-risk studies produced a similar estimate (OR 1.67, 95% CI, 1.39–2.01), supporting robustness. Nevertheless, residual confounding, differences in sequencing pipelines, variable sampling sites, inconsistent adjustment for antibiotics and ethnicity, and the predominance of observational cohorts remain important limitations. Overall, our findings agree with the referenced literature: dysbiosis and low-lactobacilli communities are associated with increased PTB risk, L. crispatus dominance is consistently protective, and L. iners has a gestational-age-dependent relationship. Compared with individual studies, our synthesis provides a broader estimate across populations and methods, while the referenced longitudinal, recurrent-PTB, cervical, and microdiversity studies identify directions for more precise prediction.[15]

Several limitations warrant consideration. First, substantial heterogeneity (I^2=67%) was observed across studies, reflecting differences in populations, methodologies, and definitions of dysbiosis. While subgroup analyses identified several moderators (geographic region, diagnostic method, timing of sampling), residual heterogeneity remains unexplained. Second, the majority of included studies were observational, precluding definitive causal inferences. While biological plausibility is strong—ascending infection and inflammation are established mechanisms of spontaneous preterm labor—randomized controlled trials are needed to establish causality and evaluate interventions. Third, most studies were conducted in high-income countries, limiting generalizability to low- and middle-income settings where preterm birth burden is highest. Fourth, variability in sampling methods (self-collected vs. clinician-collected, vaginal vs. cervical), DNA extraction protocols, sequencing platforms, and bioinformatics pipelines may have introduced measurement bias. Standardization of these methods in future studies would enhance comparability and reproducibility.[16]

Clinical implications of these findings are substantial. Vaginal microbiota screening during early pregnancy could potentially identify women at elevated preterm birth risk, enabling targeted surveillance and preventive interventions. Probiotic supplementation with L. crispatus or other protective Lactobacillus species represents a promising avenue for prevention, though randomized controlled trials are needed to establish efficacy and safety. Additionally, antibiotic treatment of bacterial vaginosis during pregnancy has yielded inconsistent results in reducing preterm birth, possibly because antibiotics disrupt protective Lactobacillus species alongside pathogenic organisms. Future interventions should focus on microbiota restoration rather than pathogen eradication alone.[17]

Future research should standardize sampling and bioinformatics, validate species- and strain-level biomarkers, include underrepresented populations, and test whether restoring a protective microbiome can safely reduce spontaneous PTB.Future research priorities include: (1) large-scale randomized controlled trials of microbiota-targeted interventions (probiotics, prebiotics, vaginal microbiota transplantation) for preterm birth prevention; (2) development of standardized, clinically validated diagnostic criteria for vaginal dysbiosis in pregnancy; (3) mechanistic studies elucidating the immunological and inflammatory pathways linking specific microbial taxa to preterm labor; (4) investigation of interactions between vaginal microbiota, cervical length, and other preterm birth predictors; (5) evaluation of cost-effectiveness of universal vaginal microbiota screening in pregnancy; and (6) studies in diverse populations, particularly low- and middle-income countries where preterm birth burden is highest.[18-20]

CONCLUSION

In conclusion, this systematic review provides compelling evidence that vaginal dysbiosis is significantly associated with increased preterm birth risk. L. crispatus dominance confers protection, while low-lactobacilli compositions and CST IV confer risk. These findings support the integration of vaginal microbiota assessment into preterm birth risk stratification algorithms and highlight the

potential of microbiota-targeted interventions for preterm birth prevention. Future research should focus on translating these findings into effective, equitable, and scalable interventions to reduce the global burden of preterm birth.

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