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The influence of the vaginal microbiota on preterm birth: A systematic review and recommendations for a minimum dataset for future research

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ABSTRACT

Objective: This systematic review aims to identify, critically appraise and summarize the results of studies examining the relationship between the vaginal microbiota and preterm birth (PTB).
Methods: We searched the electronic databases Medline, EMBASE and the Cochrane Controlled Register of Trials for studies in any language reporting on vaginal microbiota and PTB published from 1990 to November 29th, 2017. We included any study that performed lower genital tract microbiota assessment in asymptomatic pregnant women and reported on spontaneous preterm birth, with either intact or ruptured membranes.
Results: The search strategy yielded 2171 unique citations, of which nine studies were eligible for inclusion in this review. In six studies an association was found between the composition of the vaginal microbiota and PTB, but findings differed between subgroups, ethnicities and degree of risk of PTB. In three studies no association was found. Two of these studies found a significant difference in richness and Shannon diversity between term and PTB.
Conclusions: We have demonstrated that there is a paucity of molecular based, culture-independent studies that analyse the relationship between the vaginal microbiota and PTB as an outcome. The heterogeneity precluded a meta-analysis. Studies provide contradictory evidence and the quality of the clinical information in the studies is poor. To improve quality of future studies we have provided a database of essential and desirable items of quality that are method and topic specific.

1. Introduction

Globally, preterm birth (PTB) is the major cause of neonatal mortality and morbidity and is a huge cost burden on healthcare [1]. The etiology of PTB is multifactorial albeit that the final end common pathway is the same. Infection and/or inflammation is a major cause [1–3] in up to 40% of cases of PTB [2,4] and probably much greater in early gestations where mortality and morbidity are at their greatest [5,6]. Pregnant women with vaginal dysbiosis due to bacterial vaginosis (BV), before 20 weeks of gestation, have a 5-fold increased risk of

Abbreviations: BV, Bacterial Vaginosis; BVAB, Bacterial Vaginosis Associated Bacterium; CST, Community State Types; OTU, Operational Taxonomic Units; PCR, Polymerase Chain Reaction; PPROM, Preterm Prelabour Rupture of the Membranes; PTB, Preterm Birth; SDI, Shannon Diversity Index

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late miscarriage or PTB before 34 completed weeks of gestation [7] and a 7-fold increased risk if BV is detected before 16 weeks [8]. In these women, the use of clindamycin before 22 completed weeks of gestation is associated with an 80% reduction in the rate of late miscarriage and a significant 40% reduction in PTB [9,10].

Recently, using culture-independent, molecular-based techniques, new information has become available with respect to the vaginal microbiota in health and disease [11]. These techniques have demonstrated that the genital tract microbiota is composed of different subtypes or community state types (CSTs) and show marked differences in diversity and relative abundance of dominant organisms [12]. The vaginal microbiota can be classified into five CSTs based on the dominant species: CST I (*Lactobacillus crispatus*), CST II (*Lactobacillus gasseri*), CST III (*Lactobacillus iners*), CST IV (diversity group) and CST V (*Lactobacillus jensenii*) [12,13]. We have searched for the evidence pertaining to the vaginal microbiota and subsequent PTB using a systematic review. The aims of our study was: i) to review the quality of data of the included studies and the diagnostic methods used and ii) the association between specific microbiota findings and the risk of PTB; with a view to providing guidance for future research in this field with respect to molecular diagnostic methods and interventions to prevent PTB, such as antibiotics, probiotics, cervical cerclage, vaginal pessary and progesterone.

2. Methods

We conducted the systematic review according to the recommendations of the MOOSE group [14] and reported the review in accordance with the PRISMA Statement [15].

2.1. Search strategy

A medical information specialist (J.L.) performed a comprehensive search in OVID MEDLINE, OVID EMBASE, and the Cochrane Controlled Register of Trials (CENTRAL) from 1990 to November 29th, 2017 without any language restrictions. We used 1990 as starting point given the novelty of culture-independent, molecular-based techniques. We used the following search terms, adapted to each database: microbiota or bacterial vaginosis and adverse pregnancy outcomes (See Appendix 1 for complete search strategies). The records retrieved were imported and deduplicated in Endnote ×7.5. The cited and citing references of the included studies were screened for additional relevant publications.

2.2. Selection criteria

We defined the following eligibility criteria for studies to be included in the review:

2.2.1. Types of studies

We included observational cohorts, case-control or cross-sectional studies that assessed the lower genital tract microbiota using culture-independent molecular-based techniques and reported on subsequent spontaneous PTB and late miscarriage. We excluded case reports and case series without a control group of term deliveries and studies that analysed the effects of interventions, such as vaginal pessary and cervical cerclage, on the lower genital tract microbiota or on PTB. Since the effect of vaginal micronized progesterone on the vaginal microbiota is unknown, studies that used vaginal micronized progesterone were excluded. Studies in which women with a previous PTB were treated with weekly intramuscular progesterone were included.

2.2.2. Types of participants

We included studies that collected vaginal microbiota samples from asymptomatic pregnant women, both nulliparous and multiparous, at any gestational age, of any ethnicity, age, and socio-economic background. We included studies that enrolled women at low risk for PTB as well as those categorized as high risk due to previous PTB or other risk factors such as short cervical length. Studies in women with signs of a threatened PTB were included, provided sampling of the vaginal microbiota was performed when these women were asymptomatic. Studies that performed lower genital tract microbiota assessment in symptomatic women with contractions, preterm prelabour rupture of membranes (PPROM), or signs of labour were excluded.

2.2.3. Types of outcome measures

We included studies that reported on late miscarriage at 16 to 24 completed weeks of gestation or spontaneous PTB before 37 completed weeks of gestation with intact or ruptured membranes. We used the Shannon Diversity Index (SDI) as a measure of diversity of species within each vaginal microbiota [16,17].

2.2.4. Types of microbiota assessment

We included studies that applied methods for characterization of the vaginal microbiota. We excluded studies that used species specific PCRs alone, even if quantitative, because these methods do not reflect the breadth or depth of the whole microbiota.

2.3. Data collection and quality assessment

2.3.1. Selection of studies

Three authors (M.P. and B.M.L. or I.M.) independently screened title and abstract of all unique citations and selected those that were potentially relevant for full text reading. The studies, which fulfilled the selection criteria, were included in the review. We resolved initial disagreements on the eligibility of studies by discussion between the two reviewers until consensus was reached and in case of continuing disagreement with a fourth author (R.F.L).

2.3.2. Data extraction

We created a data extraction form to collect the following data from all included studies: study design, country of origin, number of participants, main characteristics of the women (low or high risk of PTB, age, parity, race and ethnicity, socioeconomic background), gestational age at sample collection, vaginal sampling method, technique used for microbiota assessment and outcome measures. Three authors (M.P. and B.M.L. or I.M.) independently extracted data from the studies, compared their findings, resolved disagreements through discussion resulting in a single final form for each included study.

2.3.3. Quality assessment

We evaluated the methodological quality of all eligible studies by using the Newcastle Ottawa Scale, which is a tool for quality assessment of non-randomised studies to be used in a systematic review and it evaluates cohort and case-control studies separately [18]. Cohort and case-control studies were assessed by selection, comparability and outcome, with a maximum total score of 9-stars. Power was considered high if a score of ≥7-stars was achieved. In addition, we used an adapted tool that was originally developed to assess the quality of studies where nifedipine was used as a tocolytic to prevent PTB [19,20]. This tool appraises general methodological parameters of the studies (selection bias, performance bias and measurement bias) as well as items related to the specific topic of interest. We prepared a comprehensive list of 46 items that could have an impact on the quality and hence validity of microbiota studies, including both method-specific items (those that involved molecular-based studies in general) and topic-specific items (those that involved studies on lower genital tract flora and adverse outcomes such as late trimester miscarriage and PTB) (Table 1). Each quality item was subdivided into those that were thought to be “essential” and those were considered “desirable”. Essential items were defined as those that should be reported in all studies assessing the vaginal microbiota and PTB. Desirable items were defined as being important but not essential for the good quality of the study.

Table 1
Overview of included Items in quality assessment tool according to selection, performance and measurement bias. In each category, items were classified as 'essential' (top of table) or 'desirable' items (bottom of table).

QUALITY ITEMS		
Selection bias (population)	Performance bias (sampling and methodology)	Measurement bias (outcome)
Essential items		
Age	Adequate assessment of gestational age	Statement of outcome measures
Race/ethnicity	Gestational age at sampling	Definition of PTB
Parity	Single or longitudinal sampling	Indication for PTB of index pregnancy
BMI	Information on primary swab	Stratification of PTB by phenotype (eg PTB with intact membranes vs PPROM)
Smoking status	Sample location	Races/ethnicities analysed separately
History of sexually transmitted infection	Information on primers used (ensure minimal bias in amplification)	Analysis of *Lactobacilli* to species level
History of PTB	Range of bacteria covered by primers	Other interventions for PTB excluded (eg cerclage, pessary)
Information on indication of previous PTB (spontaneous/ indicated/infection-related)	Attrition recorded	
Information on included singleton/multiple pregnancy		
Exclusion of other complications in pregnancy leading to PTB		
Information on use of antibiotics before sampling		
Information on use of antibiotics after sampling		
Desirable items		
Marital status	Self-collected or physician collected samples	Other subtypes of PTB (< 34 weeks, < 28 weeks)
Socioeconomic status	Measurement of cervical length	Quantitative/qualitative analysis
Alcohol intake	Use of fetal fibronectin	
Substance use	Measurement of pH	
Gestational age of previous PTB	Simultaneous cultivation	
History of late miscarriage	Objective measurement of BV by microscopy (Amsel/Nugent)	
History of LLETZ	Consent to use specimens	
Date of last sexual intercourse prior to sampling		
Reported history of douching		

BMI: Body mass index.
BV: Bacterial Vaginosis.
LLETZ: Large loop excision of transformation zone.
PTB: preterm birth.
PPROM: preterm prelabour rupture of the membranes.

Whether items were essential or desirable was consensus-based and agreed by Members of the Preterm Birth International Collaborative (PREBIC) Biomarker Working Group (2014–2018) (www.prebicglobal.org). Based on a predefined definition for each, items of quality in each study were categorized as adequate, inadequate or not stated (Table 1). Three authors (M.P. and B.M.L. or I.M.) independently assessed the quality of each study and discussed discrepancies until consensus was reached.

2.4. Data synthesis

We aimed to combine the findings of similar studies in a meta-analysis using a random effects model and to calculate the odds ratio (OR) and the 95% confidence intervals (CI) of the association between specific microbiota findings and the risk of PTB. We did not exclude studies of inadequate quality from data synthesis.

3. Results

3.1. Literature search

The search strategy yielded 2171 unique citations, of which 146 were selected for full text reading, which led to the exclusion of 137 studies. Nine studies were included in the review [21–29]. Fig. 1 shows the PRISMA flowchart of the study selection process and reasons for exclusion. The reasons for exclusion of the individual studies are listed in Table S1.

3.2. Study characteristics

The main characteristics of the included studies are summarized in Table 2. We included four cohort studies [23,24,27,29], four case-control studies [21,22,25,28], and one cross-sectional study [26]. All studies were conducted in high-income countries, seven in the US [21–25,28,29], one in Japan [26], and one in the UK [27]. The sample size of the studies varied from 40 to 96 participants.

3.3. Study populations

Most of the studies included both low and high-risk women of PTB (defined as women with a previous history of PTB). One study included only low risk women [24] and one study did not record the degree of risk of PTB [29]. In all but one study, women of different races or ethnicities were included [21–25,27–29]. One study provided no information on the ethnicity of included women [26].

3.4. Sampling methods

In two studies a single vaginal swab was collected during pregnancy [26,29]. In one study the swab was obtained between 16 and 26 weeks of gestation [29] and in the other study, between 22 and 34 weeks of gestation, with a mean of 28 weeks [26]. In two studies, two swabs were collected in pregnancy. In the first study, a sample was taken before 16 weeks of gestation and another sample between 20 and 24 weeks [24]. In the other study a sample was taken between 20 and 24 weeks and another sample between 26 and 28 weeks [27]. In five

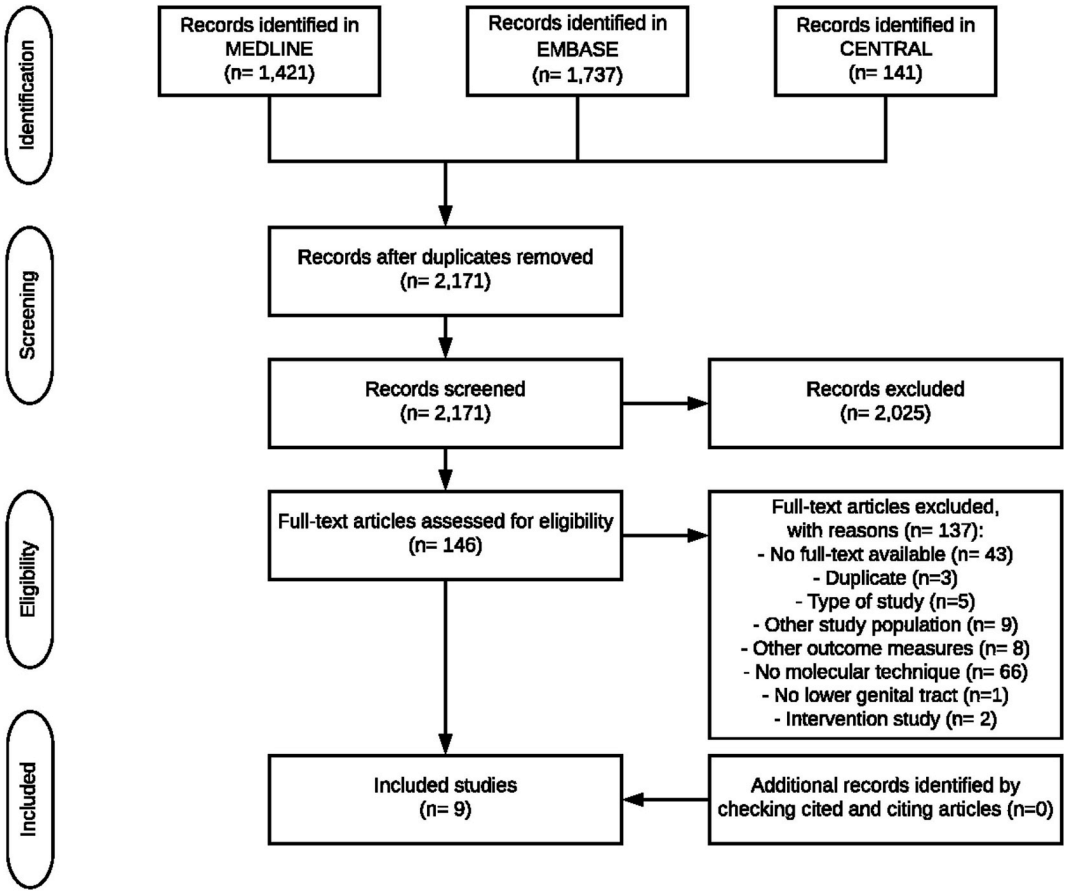

Fig. 1. Flow of the Search.

studies [21–23,25,28] vaginal swabs were collected longitudinally ranging from weekly [21,22], every 2–4 weeks [25], or if possible, in each trimester [23,28]. In six studies, vaginal samples were collected by a healthcare professional from the upper one-third of the vagina or from the posterior fornix [23,25–29] whereas three studies used patient self-collected vaginal swabs [21,22,24]. The diversity of bacteria detected in each study ranged from 25 to 2885 operational taxonomic units (OTU) (though in one study, the number of OTU's was not stated [21]) and included both BV-associated bacteria as well as *Lactobacilli.* In eight studies, bacterial DNA was extracted directly from the vaginal sample [21–28] and in one study DNA was extracted from stored Gram stain slides [29]. The extracted DNA was then pre-amplified using 16S rRNA gene universal primers.

3.5. Quality assessment

3.5.1. Newcastle Ottawa scale assessment

When using the Newcastle Ottawa Scale, three out of the five cohort studies where assessed as having high power with a score > 7-stars (Table 3a) [23,24,29]. The two studies that scored < 7-stars were primarily due to selection bias, as well as a lack of clarity as to which women were symptomatic or asymptomatic at 24–28 weeks gestation. Furthermore, these studies failed to control for confounding variables such as previous PTB, ethnicity and smoking, which were different between term and preterm groups [26,27]. Three case-control studies were determined to have high power (Table 3b) [21,25,28]. One study was assigned 6-stars due to selections bias, poor clarity in the selection procedure and in controlling for degree of risk, and reduced comparability between cases and controls [22].

3.5.2. Adapted quality assessment tool

The quality of the individual studies using our adapted quality assessment tool is summarized in Table 3c and is shown graphically in Figs S1a-c. The overall quality of "essential" items in the individual studies was graded as good (> 70%), moderate (51–70%) or poor (≤50%) respectively according to the percentage graded as adequate (See Table 1 for the quality assessment form with a list of all items). Two out of four case control studies were graded as moderate in the overall quality assessment, in which the three components of selection bias, performance bias, and measurement bias were combined, whereas one of the prospective cohort studies was graded as good [24], as well as two case control studies [22,28]. The only cross-sectional study was graded as poor [26]. The size of the studies did not influence the quality assessment, since the best and poorest study both included 40 cases and all other studies were larger and graded with moderate quality.

For the items of selection bias, three studies scored 'poor' with < 50% of all essential items scored as adequate [25,27,29]. Five studies were considered of moderate quality with respect to selection bias, with 50–70% of the items scored as adequate [21–23,26,28] and one study was considered of good quality [24]. For performance bias, one study was assessed to be of moderate quality [26] and the other eight studies of good quality [21–25,27–29]. For measurement bias, one study was of poor quality [26], two were of moderate quality [27,29], and six were of good quality [21–25,28]. One study was considered as having poor quality when assessed using both the adapted tool and the Newcastle Ottawa Scale [26].

3.6. Selection bias

Two of the studies reported no information on whether the women included had had a previous history of PTB [25,29] and only two

Table 2
Main characteristics of included studies.

Author, year, country	Study design	Number of participants	Risk assessment for PTB (low/high)	Maternal age (range)	Ethnicities studied	Gestational age at sampling (weeks)	Origin of sample	Molecular technique used
Callahan [21] (2017) USA	Case-control in two cohorts	N = 135	Mixed	17–41	White, Black, Asian, American Indian, Pacific Islander	Weekly	Midvaginal wall	V4 region of 16S rRNA
DiGiulio [22] (2015) USA	Case-control	N = 49	Mixed	19–45	White, Asian, Pacific islander, African American, Indian Black, Other	Weekly	Vaginal swab (not specified)	V3-V5 region of 16S rRNA gene
Hyman [23] (2014) USA	Prospective cohort	N = 88	Mixed	NR	African American, Asian, Non-hispanic white, Hispanic, Other	Each trimester if possible (study entry in any trimester)	Posterior fornix of the vagina	16S rRNA
Nelson [24] (2016) USA	Prospective cohort study	N = 40	Low risk	15–22	African American	1st swab: 9–16 weeks 2nd swab: 20–24 weeks	Self-collected vaginal sample	V4 region of 16S rRNA
Romero [25] (2014) USA	Nested case-control	N = 90	Mixed	20–28	African American, White, Others	Every 4 weeks until 24 weeks, then every 2 weeks	Posterior fornix of the vagina	V1-V3 of regions of 16S rRNA
Shiozaki [26] (2014) Japan	Prospective, cross-sectional	N = 41	Mixed	22–41	NR	22–34 weeks	Posterior fornix of the vagina	16SrDNA
Stafford [27] (2017) United Kingdom	Prospective cohort study	N = 80	Mixed	NR	White, Black, Asian, Mixed	20–22 weeks	Posterior vaginal fornix	V1-V3 region of 16S rRNA
Stout [28] (2017) USA	Nested case-control study	N = 77	Mixed	NR	African-American, Other	Serial swabs	Lateral side wall of the vaginal canal	V1V3 and V3V5 regions of 16SrRNA
Subramaniam [29] (2016) USA	Retrospective cohort	N = 40	NR	17–26	African American, White	21^{+0} to 25^{+6}	The mucus on the ectocervix and the posterior vaginal fornix	V4 region of 16S rDNA, universal primers

DNA: Deoxyribonucleic Acid PTB: preterm birth.
NR: not reported RNA: Ribonucleic acid.

Table 3a
Quality assessment for cohort studies using Newcastle Ottawa Scale.

Newcastle Ottawa Scale for Cohort studies										
	Selection				Comparability	Outcome			Total score	Power (> 7 is high)
	Representativeness of exposed cohort (Maximum: ★)	Selection of non-exposed cohort (Maximum: ★)	Ascertainment of exposure (Maximum: ★)	Demonstration that outcome/disease was not present at start of study (Maximum: ★)	Comparability of cohort on the basis of the design or analysis (Maximum: ★★)	Assessment of outcome (Maximum: ★)	Was follow-up long enough for outcome to occur (Maximum: ★)	Adequacy of follow up of cohort (Maximum: ★)		
Hyman [23] (2014)	★	★	★	★	★	★	★	★	8	High
Nelson [24] (2016)	★	★	★	★	★★	★	★	★	9	High
Shiozaki [26] (2014)	–	–	★	–	★	★	★	★	5	Moderate
Stafford [27] (2017)	★	★	★	–	–	★	★	★	6	Moderate
Subramaniam [29] (2016)	★	★	★	★	★	★	★	★	8	High

Table 3b
Quality assessment for case control studies using Newcastle Ottawa Scale.

Newcastle Ottawa Scale for Case Control studies										
	Selection				Comparability	Outcome			Total score	Power (> 7 is high)
	Is the case definition adequate? (Maximum: ★)	Representativeness of the cases (Maximum: ★)	Selection of controls (Maximum: ★)	Definition of controls (Maximum: ★)	Comparability of cases and controls on the basis of the design or analysis (Maximum: ★★)	Assessment of exposure (Maximum: ★)	Same method of ascertainment of cases and controls (Maximum: ★)	Non-response rate (Maximum: ★)		
Callahan (2017) [21]	★	★	★	★	★	★	★	★	8	High
DiGiulio (2015) [22]	★	–	–	★	★	★	★	★	6	Moderate
Romero (2014) [25]	★	★	★	★	★★	★	★	★	9	High
Stout (2017) [28]	★	–	★	★	★★	★	★	★	8	High

Table 3c
Quality assessment of included studies using additional tool based on selection bias, performance bias, and measurement bias.

Quality of individual studies (% of essential items adequately reported)				
	Selection bias (12 items)	Performance bias (8 items)	Measurement bias (7 items)	Overall quality (27 items)
Callahan (2017) [21]	50.0	87.5	71.4	Moderate (69.6%)
DiGiulio (2015) [22]	58.3	75.0	85.7	Good (73.0%)
Hyman (2014) [23]	58.3	75.0	71.4	Moderate (68.3%)
Nelson (2016) [24]	75.0	87.5	85.7	Good (82.7%)
Romero (2014) [25]	33.3	75.0	85.7	Moderate (64.7%)
Shiozaki (2014) [26]	58.3	62.5	14.3	Poor (45.1%)
Stafford (2017) [27]	33.3	87.5	57.1	Moderate (59.3%)
Stout (2017) [28]	50.0	100.0	85.7	Good (78.6%)
Subramaniam (2016) [29]	41.7	100.0	57.1	Moderate (66.3%)

studies gave additional information on the indication for the prior PTB (spontaneous, indicated or infection-related PTB) [23,24]. Only one study described whether other complications, such as symptoms of vaginal/cervical/urinary infection, significant vaginal bleeding and/or placenta praevia, or comorbidity in the current pregnancy leading to PTB were excluded [23]. Furthermore, only four studies reported whether women were excluded if they had used antibiotics prior to sampling [22–24,26] and only two studies recorded whether antibiotics were prescribed later in pregnancy [22,26]. In all other studies no information on these items were described.

3.7. Performance bias

When the individual items of performance bias were assessed, one of eight “essential” items was scored as poor. Only two studies adequately described the assessment of gestational age [28,29], which we consider is essential in any study in which PTB is a primary outcome.

3.8. Measurement bias

In studies using PTB as the primary outcome it is important to state the definition of PTB used. In one study this definition was lacking [26]. Furthermore, it is important to distinguish the various phenotypes of PTB, whether spontaneous preterm labour with intact membranes or PPROM, especially as infection/inflammation plays an important role. Only two studies stratified their results by the phenotype of PTB [22,25]. Only one study gave information on the use of interventions for the prevention of PTB, such as cervical cerclage, vaginal pessary or progesterone [28]. Finally, while the rate of PTB and CST types differ among racial/ethnic groups [12,30], only three of the included studies gave information about racial mix [23,24,29].

3.9. Data synthesis of vaginal microbiota and PTB

Six studies found an association between the composition of the vaginal microbiota and PTB [21–24,27,28] but the findings differed between ethnicities and degree of risk of PTB. In three studies no association was found between the composition of the vaginal microbiota and PTB (Table 4) [25,26,29].

3.9.1. Shannon diversity index

The SDI differed significantly between racial/ethnic groups [23]. The greatest species diversity was found among African American women, followed by Hispanic and women of other race. The SDI was less diverse among Asians and Caucasians. Unfortunately, other than Caucasians, the subgroups and events were too small or too few to analyse the effect of race/ethnicity on birth outcome. In Caucasians, a statistically significant difference in the average SDI was found among women with a PTB compared to women who delivered at term [23]. Another study [28] also found a significant difference in richness and SDI between term and PTB. In subjects with subsequent term delivery, the vaginal microbiota demonstrated stable community richness and SDI, whereas subjects with subsequent PTB had significantly decreasing vaginal richness, diversity, and consistency during pregnancy ($P < 0.01$). This change occurred between the first and second trimester [28]. However, other studies found no significant difference in the SDI between the preterm and term group [24,25], though, based on the phylogenetic diversity and Chao1 (another index of α-diversity reflecting the diversity of species with each woman's vaginal microbiota), a lower diversity in the vaginal microbiota of women who delivered preterm compared with term was suggested, though these findings were not statistically significant ($p = 0.077$ and $p = 0.066$, respectively) [24]. Instead of SDI, one study used Simpson's Inverse Diversity Index rather than SDI, and found no significant differences between term and PTB [27].

3.9.2. Community state type

One study found in their mixed population of women at low and high risk of PTB, that women with a *Lactobacillus*-depleted CST type IV had a strong association with PTB [22]. A dose-response, as well as a temporal relationship, was found with further risk stratification for women with CST IV and higher abundance of *Gardnerella* spp or *Ureaplasma* spp. This correlation remained significant after adjusting for white or non-white race [22]. The proposed associations between PTB and lower *Lactobacillus* and higher *Gardnerella* abundances were replicated in another study, but only in the low-risk cohort (predominantly Caucasian women), not in the high-risk cohort (predominantly African American women) [21]. These studies also found that *L. crispatus* was associated with a low risk of PTB in both cohorts, while *L. iners* was not, and that a subspecies clade of *G. vaginalis* explained the genus association with PTB [21]. However, in an African-American cohort, no significant differences in *Gardnerella* or *Ureaplasma* abundance were found in women delivering term or preterm [28]. Another study showed that women with a PTB had mostly CST III at the 9–16 weeks collection point. No difference in the abundance of *Lactobacillus* spp. or *Gardnerella* spp. was found between the term and PTB, but among women who delivered preterm there were lower levels of *Coriobacteriaceae*, *Sneathia*, *Prevotella*, and *Aerococcus* [24]. One of the most recent studies found that at 20–22 weeks, the women who delivered at term had a proportion of CST I (*L. crispatus* dominated microbiota) > 2-fold higher compared to women with a PTB. Furthermore, CST V (*L. jensenii* dominated microbiota) at 20–22 weeks was > 2-fold lower in the term group compared to women with a PTB [27]. In contrast, another study found no differences in the frequency of different CSTs between women who delivered at term and those with PTB [25].

Table 4
Main Results per study on Vaginal Microbiota and Preterm Birth.

Author, year	Main results on vaginal microbiota and preterm birth
Callahan [21] (2017)	Previously proposed associations between PTB and lower *Lactobacillus* and higher *Gardnerella* abundances replicated in the low-risk cohort, but not in the high-risk cohort *Lactobacillus crispatus* was associated with low risk of PTB in both cohorts, while *Lactobacillus iners* was not, and that a subspecies clade of *Gardnerella vaginalis* explained the genus association with PTB Our findings extend and corroborate the association between the vaginal microbiota and PTB, demonstrate the benefits of high-resolution statistical bioinformatics in clinical microbiome studies, and suggest that previous conflicting results may reflect the different risk profile of women of black race
DiGiulio [22] (2015)	Microbiota community taxonomic position and diversity remained stable during pregnancy Prevalence of a *Lactobacillus*-poor vaginal community state type (CST-IV) was inversely correlated with gestational age at delivery Risk for PTB was more pronounced for subjects with CST-IV accompanied by higher abundance of *Gardnerella* or *Ureaplasma* abundances
Hyman [23] (2014)	Species diversity was greatest among African Americans Change in microbiome/Lactobacillus content and presence of putative novel/noxious bacteria did not correlate with PTB There was a statistically significant difference in the average SDI between Caucasian women who had a PTB and who carried to term
Nelson [24] (2016)	We found lower bacterial diversity with lower abundance of *Coriobacteriaceae*, *Sneathia*, *Prevotella*, and *Aerococcus* compared with women delivering at term The Shannon diversity index was not significantly different between the groups A lower diversity in the vaginal microbiota of women delivered preterm compared with term was suggested, but these findings were not significantly different
Romero [25] (2014)	The composition of the vaginal microbiota during normal pregnancy changed as a function of gestational age, with an increase in the relative abundance of four *Lactobacillus* spp and decreased abundance of anaerobe or strict-anaerobe microbiota as pregnancy progressed No bacterial taxa differed in relative abundance between women who had a spontaneous PTB and those who delivered at term No differences in the frequency of the vaginal community state types (CST I, III, IV-B) between PTB and term birth were detected
Shiozaki [26] (2014)	There were no significant differences in vaginal microbiota among term deliveries, women with preterm labour symptoms but term deliveries, and PTB
Stafford [27] (2017)	At 20–22 weeks, the term-delivered women indicated a proportion of CST1-dominated microbiota > 2-fold higher compared to the preterm-delivered women CSTV was > 2-fold higher in the preterm group compared to term women at 20–22 weeks The SDI shows no significant difference between any of the sample groups
Stout [28] (2017)	In subjects with subsequent term delivery, the vaginal microbiome demonstrated stable community richness and Shannon diversity, whereas subjects with subsequent preterm delivery had significantly decreased vaginal richness, diversity, and evenness during pregnancy This change occurred between the first and second trimesters Within-subject comparisons across pregnancy showed that preterm birth is associated with increased vaginal microbiome instability compared to term birth No distinct taxa were associated with preterm birth
Subramaniam [29] (2016)	Microbiota did not differ by race or birth timing, but there was a nonsignificant association between certain microbial clusters and PTB

CST: Community state type.
PTB: Preterm birth.
SDI: Shannon diversity index.
Spp: Species.

4. Discussion

4.1. Main findings

In this systematic review we have demonstrated that there is a paucity of molecular based culture-independent studies with good quality clinical information that analyse the relationship between the vaginal microbiota and PTB as an outcome. Overall, the available studies provided contradictory evidence but the more recently published studies provide an association between a vaginal dysbiotic microbiota and PTB.

As pregnancy progresses, the genital tract microbiota becomes progressively more dominated by *Lactobacilli* such that by term, the vaginal microbiota poses less threat to the fetus as it passes through the birth canal [31]. If screening for vaginal dysbiosis as a predictor for infection-related PTB begins at gestations beyond 24 weeks, the opportunity to prevent late miscarriage and extreme PTB is lost. The earlier in pregnancy that vaginal dysbiosis, in the form of BV is diagnosed, the greater is the risk of PTB [10]. Nevertheless, 83% of women with vaginal dysbiosis at a mean of 16 weeks gestation will still deliver beyond 34 completed weeks of gestation [7]. Accordingly, more information is needed about vaginal dysbiosis and eubiosis, if we are to distinguish between those women with vaginal dysbiosis who are likely to have an adverse outcome of pregnancy compared to those who will not, so that better interventions can be developed. While recent advances in cultivation-independent techniques indicate that an increase in diversity in the gut microbiota is associated with a decreased susceptibility to disease, the opposite appears to be the case for the vaginal microbiota [11,12,32].

4.2. Molecular-based, culture-independent techniques

4.2.1. Vaginal eubiosis

This is normally achieved through vaginal colonisation by a beneficial lactic acid producing microbiota, predominantly from the genus *Lactobacilli*. New information from molecular-based, culture-independent techniques, has established that, worldwide, the healthy vagina is colonised by one, or at the most, two *Lacobacillus* spp from a shortlist of four: *L. crispatus* L. *gasseri, L. iners,* and *L. jensenii,* corresponding to CSTs I, II, III and V respectively [11]. This information has implications for the development of potentially vaginal-specific probiotics.

4.2.2. Vaginal dysbiosis

Though molecular based diagnostic tests exist and have been licensed for use [33] the current gold standard for diagnosis of BV, particularly for research purposes, remains Nugent scoring on Gram stain microscopy. There are also previously under detected and hence underappreciated organisms like *Atopobium vaginae* that contribute to vaginal dysbiosis. Accordingly, this systematic review aimed to explore the possibility that, with new information from molecular based studies, the vaginal microbiota might be predictive of PTB.

4.3. Quality assessment tool

We found a paucity of studies that were methodologically acceptable and of those that were acceptable, the quality of the clinical

information was poor. The results of the studies were inconsistent though there was a trend towards an association between the vaginal microbiota and PTB with more recent studies and with better quality, implying a greater understanding of the problem and the technology required to demonstrate an association. Since there is a considerable ethnic and geographic variation in the composition of the vaginal microbiota, the inconsistencies of the results may be due to the heterogeneity of the populations studied.

4.3.1. Comparison between the quality assessment tools

The articles were evaluated slightly differently depending on which quality assessment tool was used. When applying our own adapted assessment tool, only three studies [22,24,28] were assessed as having good quality, whereas 7 out of 9 studies were assessed as having high quality when applying the Newcastle Ottawa Scale [21,23–25,27–29]. This difference is explained by our adapted tool being more specific, more detailed, and having higher requirements, so we feel this is a strength of this review.

The risks associated with a specific vaginal microbiota and PTB may not be due the presence of dysbiosis but the absence of eubiosis, or something in between. One of the reasons why more recent studies have found a trend towards an association between the vaginal microbiota and PTB may be the appreciation of the role of *Lactobacillus* species in general and *L. iners*, in particular. The existence of *L. iners* was not known before 1999, as it does not grow on the selective media regularly used for culture of *Lactobacilli*. Accordingly, its role in vaginal eubiosis and dysbiosis has been underappreciated. In contrast to *L. crispatus*, which is rarely present in BV, *L. iners* can be detected in high abundance in many women with and without BV, and in some studies, *L. iners* was the only species of *Lactobacilli* detected in women with BV. This may be because *L. iners* is better adapted to the conditions associated with BV, such as the elevated pH and the polymicrobial state of the vaginal microbiota [11]. Alternatively, the observations could result from the relative resistance of *L. iners* to unknown factors, which lead to the demise of other species of *Lactobacilli* during the development of BV. Finally, unlike *L. crispatus*, there may be a relative lack of antagonism of *L. iners* to the BV-associated anaerobes, so that their dominance predisposes the individual to the acquisition of BV or a BV-associated CST-IV.

4.4. The relationship between L. iners and PTB

In a low risk population of pregnant women, with eubiosis in early pregnancy, the dominant species of vaginal *Lactobacilli* were studied, with particular reference to the diversity of species of *Lactobacilli* in women who had a term birth or a PTB [34]. *L. iners* alone was detected in 85% of women who had a PTB compared to only 16% of women who delivered at term ($p < 0.001$). Conversely, 56% of women who delivered at term and only 8% of women who had a PTB had two or more species of *Lactobacilli* in the vagina. These data suggest that the risk of PTB may not be due to the presence of *L. iners*, but may be due to either: i) the lack of a protective effect of *L. iners* when it is the sole species of *Lactobacilli* in the vagina or ii) *L. iners*, in combination with other species of *Lactobacilli*, does not influence the vaginal microbiota or impair the protective effect of lactic acid producing lactobacilli. Accordingly, the absence of species of *Lactobacilli* other than *L. iners*, or very low diversity in species of *Lactobacilli* with a protective role against potential pathogens in early pregnancy, may increase the potential for PTB.

Though not included in our review, a recent study has provided important information about the role of *L. iners* and PTB [35]. A *L. iners* dominated microbiota was significantly overrepresented in women who delivered early preterm (67%), compared to those who delivered late preterm (31%), or at term (29%), ($p = 0.003$). Conversely, a *L. crispatus* dominated microbiota was associated with subsequent term birth (46%) compared with early PTB (11%) ($p = 0.009$), and a comparatively longer duration of pregnancy than that associated with *L. iners*. The accuracy of prediction of PTB using CST grouping at 16 weeks gestation, was associated with a sensitivity and specificity that was comparable to the use of transvaginal ultrasound for the measurement of cervical length. *L. iners* dominance predicted early PTB with a sensitivity of 67% and a specificity of 71% and a PPV of 22%, while the absence of *L. iners* was associated with a NPV of 94%. *L. crispatus* in high abundance was strongly predictive of birth > 34 completed weeks of gestation (specificity = 89% and PPV = 97%) [35].

L. crispatus has advantages over *L. iners* with respect to the chirality ratio between the production of the D- and L-isomer of lactic acid, and whether or not the lactic acid molecule is protonated or dissociated as the negatively charged anion. All of these have major functional implications [36]. This may be why studies based on culture or microscopy where species of *Lactobacilli* could not be identified concluded that it was BV rather than the species of *Lactobacilli* that conferred risk [35]. The smaller genome size of *L. iners* is similar to that of human symbionts and parasites while facultative symbionts have intermediate genome sizes. Accordingly, under some circumstances, *L. iners* could be a genuine vaginal symbiont, but also a potential opportunistic pathogen. Women with high levels of *L. iners,* can be either BV-positive or BV-negative and have either high or low pH. This adaptation to the vaginal econiches, with the genital mycoplasmas [37,38], is not only due to the presence or absence of the organism, but also the quantity, or relative abundance.

4.5. Choice of primers

Next-generation sequencing methods are used extensively in studies of the vaginal microbiota using partial 16SrRNA gene amplicons. The choice of primers used for studying the vaginal microbiota has major implications on the biological evaluation of the results. Primers spanning the V3/V4 region identify a greater number of taxa in the vaginal microbiota than those of the V1/V2 region, particularly, taxa such as *Gardnerella vaginalis*, *Bifidobacterium bifidum* and *Chlamydia trachomatis*. Several species that influence vaginal eubiosis and dysbiosis, are not represented in V1/V2-based community profiles. Accordingly, missing or underestimating the frequency of these species overestimates the abundance of other taxa and fails to assess the vaginal bacterial diversity. Accordingly, it is important that these taxa are sought using the V3/V4 region for the assignment of CSTs [39].

5. Conclusion

Despite the need to take advantage of new information from molecular-based, cultivation independent studies and the role of the vaginal microbiota in spontaneous labour and PTB, the current evidence is limited, clinical data is of poor quality and results are contradictive. Of the three studies that failed to show an association between the composition of the vaginal microbiota and PTB (Table 4) [25,26,29] two were published in 2014, and one in 2016. In contrast, of the six studies that found an association between the composition of the vaginal microbiota and PTB [21–24,27,28], one of each was published 2014, 2015, and 2016, while the other three were published in 2017. The reason why the more recently published studies provide evidence of an association between a dysbiotic microbiota and PTB may be due to a better understanding of the role of *L. iners* in vaginal eubiosis and dysbiosis. Among our recommendations for a minimum data set, we have stated that future studies should address the role of *L. iners* specifically. In addition, if data exists, previous studies that failed to show an association between the composition of the vaginal microbiota and PTB should reanalyse their data with the role of *L. iners* in mind. Given the novelty of the subject/technique and the expected growing number of studies, together with the heterogeneity of the studies and the clinical data that lacks comparability between studies, we recommend a minimum dataset of essential and desirable, method-specific and topic-specific items of quality that should be applied to all studies addressing the relationship between the vaginal microbiota and PTB.

Finally, when including previous PTB as a risk factor for subsequent PTB in association with vaginal dysbiosis, efforts should be made to

establish whether or not the previous PTB was infection-related.

Contribution to authorship

MJCSP, BML, IdM, RFL, and RM conceived the idea for the study. JL performed the search in literature. MJCSP, BML, IdM performed the data collection. MJCSP, BML, IdM, PJH and RFL wrote the first draft. JSJ provided molecular microbiological advice. All authors interpreted the data, revised the article, and approved the final version.

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Disclosure

The authors report no conflict of interest.

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Appendix A. Supplementary data

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