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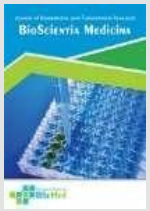

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Adjunctive Vaginal Probiotic Therapy for Preterm Premature Rupture of Membranes: A Systematic Review and Meta-Analysis of Latency Period, Maternal Infection, and Neonatal Morbidity

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A B S T R A C T

Background: Preterm premature rupture of membranes (PPROM) significantly drives preterm birth rates and consequent neonatal morbidity and mortality. While standard antibiotic therapy aims to prolong pregnancy latency, it concurrently disrupts the protective vaginal microbiota. Adjunctive vaginal probiotics have been investigated as a means to restore beneficial flora, potentially mitigating ascending infection and improving perinatal outcomes. This study systematically synthesized the current randomized trial evidence regarding this adjunctive therapeutic strategy. **Methods:** We performed a systematic review and meta-analysis following PRISMA guidelines. PubMed, EMBASE, and CENTRAL databases were searched (2014–October 2025) for randomized controlled trials (RCTs) comparing adjunctive vaginal probiotics plus antibiotics versus antibiotics (alone or with placebo) in singleton pregnancies complicated by PPROM between 24+0 and 34+0 weeks' gestation. Primary outcomes included the latency period (days) and maternal chorioamnionitis or infectious morbidity. Key secondary outcomes were neonatal intensive care unit (NICU) admission, neonatal sepsis, and neonatal mortality. Data were pooled using a random-effects model, calculating Mean Differences (MD) or Risk Ratios (RR) with 95% Confidence Intervals (CI). Risk of bias was assessed using the Cochrane RoB 2 tool. **Results:** Three RCTs, encompassing 330 participants, met the inclusion criteria. Significant methodological limitations, including high risk of bias and critical baseline confounding by gestational age in the largest trial, were identified across the included studies. A sensitivity analysis addressing high heterogeneity (I^2=98%) for latency (excluding one retrospective study; n=290) indicated a modest but statistically significant prolongation associated with probiotics (MD 2.98 days; 95% CI 1.80–4.16; p<0.0001; I^2=0%). Probiotic use was linked to a significantly lower risk of maternal infection (RR 0.43; 95% CI 0.24–0.77; p=0.005; I^2=0%; n=270). Statistically significant reductions were also observed for NICU admission (RR 0.59; 95% CI 0.46–0.75; p<0.0001; I^2=55%; n=330) and neonatal mortality (RR 0.38; 95% CI 0.18–0.81; p=0.01; I^2=0%; n=270), although these estimates are likely inflated due to baseline confounding. **Conclusion:** This meta-analysis suggests adjunctive vaginal probiotics may offer benefits in PPROM management by modestly prolonging latency and significantly reducing maternal infectious morbidity. While substantial reductions in NICU admission and neonatal mortality were observed, these findings must be interpreted with extreme caution due to the limited quantity and low quality of the primary evidence, particularly the high risk of bias and confounding. Definitive conclusions cannot be drawn, and routine clinical adoption is not supported by current evidence. High-quality, large-scale RCTs are imperative.

1. Introduction

Preterm birth, defined as delivery before 37 completed weeks of gestation, remains a paramount challenge in global perinatal health, representing the single largest cause of mortality in children under five years old.[1] Beyond the tragic loss of life exceeding one

million infants annually, the approximately 15 million babies born preterm each year face a significantly elevated risk of acute neonatal complications and long-term sequelae. Immediate challenges often include respiratory distress syndrome (RDS) due to lung immaturity, intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), and sepsis, stemming from underdeveloped organ systems and compromised immunity.[2] The long-term trajectory for survivors can be marked by neurodevelopmental disabilities like cerebral palsy, cognitive deficits, chronic respiratory disease, and an increased predisposition to metabolic and cardiovascular conditions later in life. This substantial burden underscores the urgent need for effective strategies to prevent preterm birth and mitigate its consequences. Within this complex syndrome, preterm premature rupture of the fetal membranes (PPROM)—the spontaneous rupture of the amnion and chorion prior to 37 weeks' gestation and before labor onset—constitutes a particularly critical obstetric emergency. Although PPROM complicates only about 3% of pregnancies, it serves as the direct antecedent event in a disproportionately large fraction, roughly one-third, of all preterm deliveries. The clinical course following PPROM is dictated by a precarious balance: the need to prolong gestation to allow for crucial fetal maturation in utero, versus the rapidly escalating risk of microbial invasion of the amniotic cavity (intrauterine infection or chorioamnionitis).[3] This infection poses significant threats of sepsis and inflammatory injury to both the mother and the fetus. Expectant management, the standard approach between fetal viability and 34 weeks, aims to maximize gestational age. However, inherent biological pressures often lead to spontaneous labor within a week of membrane rupture, frequently truncating the latency period needed for interventions like antenatal corticosteroids to achieve their full protective effect.

Understanding the pathophysiology leading to PPROM necessitates appreciating the profound influence of the vaginal microbiome on reproductive tract health and pregnancy maintenance. While PPROM is multifactorial, compelling evidence implicates ascending microbial infection and the resultant inflammation as a primary and well-characterized pathway driving premature membrane weakening and rupture.[4] This pathological cascade frequently originates from disturbances within the vaginal microbial ecosystem. A healthy vaginal environment during pregnancy (eubiosis) is typically characterized by low bacterial diversity and the conspicuous dominance of specific *Lactobacillus species*, such as *Lactobacillus crispatus*, *Lactobacillus gasseri*, or *Lactobacillus jensenii*.[5] These commensal bacteria actively maintain homeostasis through several key protective mechanisms. Their fermentation of glycogen produces high concentrations of lactic acid, creating an acidic milieu ($pH \leq 4.5$) that intrinsically inhibits the proliferation of most opportunistic and pathogenic microbes. Furthermore, these lactobacilli produce antimicrobial compounds like hydrogen peroxide and bacteriocins, compete for epithelial adhesion sites, and modulate local immune responses to prevent excessive inflammation. Advanced molecular techniques classify these healthy profiles predominantly as Community State Types (CSTs) I (*L. crispatus* dominant), II (*L. gasseri* dominant), or V (*L. jensenii* dominant), with CST I being most strongly associated with optimal pregnancy outcomes. It is crucial to recognize that significant functional differences exist even between strains within the same *Lactobacillus* species—strain specificity dictates the potency of acid production, antimicrobial activity, adherence capabilities, and immunomodulatory potential.[6] Conversely, vaginal dysbiosis involves a depletion of these protective lactobacilli and a polymicrobial overgrowth of diverse anaerobes (*Gardnerella vaginalis, Prevotella, Atopobium vaginae*) and facultative bacteria, often corresponding to CST IV.[7] This disruption elevates vaginal pH and allows pathogens to flourish. Many dysbiosis-associated bacteria produce virulence factors, including sialidases that degrade cervical mucus and proteases (collagenases, MMPs) that directly attack the

structural integrity of the chorioamniotic membranes. This enzymatic degradation, combined with microbial PAMPs triggering a host inflammatory response (release of cytokines, prostaglandins), progressively weakens the membranes, making them susceptible to rupture under mechanical stress. *Lactobacillus* iners-dominated communities (CST III) represent an intermediate, less stable state, sometimes associated with increased risk compared to CST I.[8]

Given the pivotal role of infection, broad-spectrum antibiotic administration is a cornerstone of expectant PPROM management between 24 and 34 weeks. This intervention significantly prolongs the latency period, reduces maternal chorioamnionitis, and decreases neonatal infectious morbidity, forming an indispensable part of standard care. However, this necessary therapy introduces the "antibiotic paradox." The antibiotics (ampicillin, erythromycin) are non-selective, eradicating not only potential pathogens but also the beneficial resident *Lactobacillus* populations. This iatrogenic disruption plunges the vaginal ecosystem into a state of profound dysbiosis. The continuous leakage of neutral-pH amniotic fluid further exacerbates this by buffering the vaginal canal and washing away remaining microbes, hindering the re-establishment of protective acidity. This antibiotic-induced ecological disruption can inadvertently create opportunities for the overgrowth of intrinsically resistant bacteria (Enterococci) or opportunistic fungi (Candida species), potentially leading to secondary infections or inflammation that counteract the initial benefits of antibiotic therapy. Mitigating this collateral ecological damage is therefore a key consideration in optimizing PPROM management.[9]

The challenge posed by the antibiotic paradox has prompted exploration of adjunctive strategies. Vaginal probiotics, consisting of live, characterized strains of beneficial *Lactobacillus*, offer a biologically plausible approach to actively restore and maintain vaginal eubiosis during and after antibiotic treatment in PPROM. The therapeutic hypothesis centers on "ecosystem restoration," aiming to: Rapidly Re-establish *Lactobacillus* Dominance: Introduce high concentrations of exogenous lactobacilli (L. acidophilus, L. casei rhamnosus) to quickly occupy the niche cleared by antibiotics, preventing pathogen overgrowth; Restore Protective Acidity: Enable the introduced strains to produce lactic acid, re-acidifying the vagina to inhibit pathogen proliferation despite amniotic fluid leakage; Provide Direct Antagonism: Utilize the strain-specific abilities of probiotics to produce H_2O_2 or bacteriocins and competitively exclude pathogens from epithelial binding sites; Modulate Inflammation: Potentially dampen the excessive pro-inflammatory cascade triggered by infection or membrane rupture through direct interactions with the host mucosal immune system. By achieving these goals, adjunctive probiotics could theoretically synergize with antibiotics, breaking the cycle of dysbiosis, infection, and inflammation. This could lead to a safer prolongation of the latency period, reduced maternal infectious morbidity, and improved neonatal outcomes through enhanced in utero maturation and reduced inflammatory exposure. However, success depends on selecting effective strains capable of surviving antibiotic exposure and colonizing the unique post-PPROM vaginal environment.[10]

The primary novelty of this systematic review and meta-analysis lies in its exclusive and focused evaluation of vaginal probiotics used as an adjunctive therapeutic intervention implemented during the active management phase for women already diagnosed with established PPROM. This precise focus strategically distinguishes the current synthesis from the considerably larger and more heterogeneous body of literature examining the use of probiotics (often administered orally, sometimes initiated pre-conceptionally or early in gestation) for the primary prevention of preterm birth in general or high-risk, yet asymptomatic, pregnant populations. By meticulously identifying, appraising, and pooling data solely from RCTs that directly address this specific, targeted clinical question—the co-administration of vaginal probiotics alongside standard antibiotics after PPROM has occurred—this study provides the first

comprehensive, quantitative synthesis of evidence specifically tailored to inform clinical understanding and potential decision-making regarding this particular PPROM management strategy. The primary objective of this systematic review and meta-analysis was to quantitatively synthesize all available evidence from randomized controlled trials to determine the aggregate effect of adjunctive vaginal probiotic therapy (compared to standard antibiotic therapy plus placebo or antibiotics alone) on: (1) the duration of the latency period (primary outcome), (2) the incidence of maternal infectious morbidity (specifically chorioamnionitis or related composite measures; primary outcome), and (3) a predefined set of critical neonatal outcomes, including admission to the Neonatal Intensive Care Unit (NICU), incidence of neonatal sepsis, and neonatal mortality (secondary outcomes), in patients undergoing expectant management for PPROM diagnosed between 24+0 and 34+0 weeks' gestation.

2. Methods

This investigation was conducted as a systematic review and meta-analysis, adhering rigorously to established best practices for evidence synthesis. The methodology and reporting follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement, ensuring transparency and completeness. The study protocol, outlining the objectives, search strategy, inclusion criteria, outcome measures, and analysis plan, was developed a priori. Studies were systematically evaluated for inclusion based on the following predefined criteria, structured according to the PICOS framework: Population (P): Pregnant women carrying a singleton fetus who were diagnosed with PPROM between 24 weeks and 0 days (24+0) and 34 weeks and 0 days (34+0) of gestation. PPROM diagnosis required confirmation through standard clinical methods (sterile speculum examination, positive nitrazine test, positive ferning test). Studies involving multiple gestations, PPROM outside the specified gestational age window, or preterm labor with intact membranes were excluded. Intervention (I): Administration of a vaginal probiotic containing any characterized *Lactobacillus* strain(s), at any dosage or duration, specifically as an adjunct to a concurrent standard-of-care antibiotic regimen for PPROM management. Studies evaluating oral probiotics or probiotics administered without concomitant antibiotics were excluded. Comparator (C): Receipt of the standard-of-care antibiotic regimen plus an identical vaginal placebo, or receipt of the standard-of-care antibiotic regimen alone. Outcomes (O): Studies needed to report data on at least one of the following outcomes: Primary Outcomes: 1) Latency period (days from PPROM diagnosis to delivery). 2) Maternal Chorioamnionitis/Infectious Morbidity (clinical or histological diagnosis, or a composite including sepsis/endometritis, or use of inflammatory markers as a direct proxy); Secondary Outcomes: 1) NICU Admission (proportion admitted). 2) Neonatal Sepsis (suspected or proven). 3) Neonatal Mortality (death before hospital discharge). 4) Mean Birth Weight (grams). 5) 5-Minute Apgar Score. Study Design (S): Only Randomized Controlled Trials (RCTs) were eligible for quantitative synthesis. Non-randomized studies were excluded from the meta-analysis.

A comprehensive search strategy was designed and executed to identify all potentially eligible studies published from January 1st, 2014, through September 1st, 2025. This date range was chosen to capture contemporary research reflecting current PPROM management and probiotic formulations. Four major electronic databases were systematically searched: PubMed (MEDLINE), EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), and Scopus. The search strategy, developed by two independent reviewers, combined database-specific controlled vocabulary (MeSH, Emtree) with relevant free-text keywords covering PPROM, vaginal probiotics (*Lactobacillus*, specific species), adjunctive therapy, and randomized trials. Sensitivity was prioritized. An illustrative PubMed search strategy is provided in the original methods section. No language restrictions

were imposed during the search phase. Additionally, reference lists of included studies and relevant systematic reviews were manually screened to identify any potentially missed publications (backward citation chasing). A formal search for unpublished studies or grey literature was not undertaken. Study selection was performed independently by two reviewers using a two-stage process facilitated by reference management software (Zotero) for duplicate removal and screening organization. Title/Abstract Screening: All unique records were independently screened based on title and abstract; clearly irrelevant studies were excluded. Full-Text Review: Full texts of potentially eligible records were retrieved and independently assessed against the detailed PICOS criteria for final inclusion. Reasons for exclusion were documented. Disagreements at either stage were resolved by discussion and consensus or, if needed, adjudication by a third senior reviewer. Data extraction from included studies was performed independently by the same two reviewers using a pre-piloted, standardized form in Microsoft Excel. Extracted data included study identifiers, population characteristics (sample size, baseline GA), intervention details (probiotic strain, dose, duration), comparator details, and quantitative outcome data (events/total for dichotomous outcomes; mean, SD, N for continuous outcomes). Data consistency was verified by cross-checking the independent extractions, with discrepancies resolved by consensus.

The methodological quality and potential for bias in each included RCT were independently evaluated by two reviewers using the Cochrane Risk of Bias 2 (RoB 2) tool. This tool assesses bias across five domains: randomization process, deviations from intended interventions, missing outcome data, outcome measurement, and selective reporting. Each domain received a judgment ("Low risk," "Some concerns," or "High risk"), leading to an overall risk of bias judgment for the study. Particular attention was paid to the adequacy of randomization and allocation concealment, blinding procedures, and baseline comparability, especially regarding gestational age at PPROM. The retrospective nature of the Kavak et al. study led to an a priori overall rating of "High risk." Disagreements were resolved by discussion. The potential impact of bias was considered during interpretation and sensitivity analyses.

Quantitative synthesis (meta-analysis) was conducted using Review Manager (RevMan) Version 5.4. Risk Ratios (RR) with 95% Confidence Intervals (CI) were calculated for dichotomous outcomes. Mean Differences (MD) with 95% CIs were calculated for continuous outcomes. A random-effects model (DerSimonian and Laird inverse-variance method) was used for all pooled analyses due to anticipated heterogeneity in interventions and populations. Heterogeneity was assessed using the I^2 statistic (interpreted as Low: <25%, Moderate: 25-75%, High: >75%) and the Chi-squared test ($p < 0.10$ indicating significant heterogeneity). Outcomes highly sensitive to the severe baseline GA confounding in the Asif et al. study (birth weight, Apgar scores) were not pooled. Their results were presented descriptively. A planned sensitivity analysis was performed for the latency period outcome (which showed $I^2 > 75\%$) by excluding the Kavak et al. study (retrospective design, outlier effect) and pooling the remaining two prospective RCTs. Subgroup/Meta-Regression, Not feasible due to the small number (N=3) of included studies. Publication Bias, Not formally assessed due to insufficient number of studies (<10). Statistical Significance, A p-value < 0.05 was considered statistically significant for pooled effect estimates.

3. Results

The systematic search across four electronic databases yielded 348 citations. After the removal of 138 duplicates, 210 unique records underwent title and abstract screening. During this initial phase, 195 records were excluded as they did not meet the basic eligibility criteria (review articles, prevention focus, non-RCT, wrong intervention/population). Full texts were retrieved for the remaining 15 potentially relevant articles. Following detailed assessment

against the PICOS criteria, 12 of these were excluded: four due to non-randomized designs, three for investigating oral probiotics, three for enrolling incorrect populations (preterm labor with intact membranes), and two because only abstract data were available. Ultimately, this rigorous selection process identified three studies that fully met the inclusion criteria for this systematic review and meta-analysis. The PRISMA 2020 flow diagram visually summarizes this process, is provided in Figure 1.

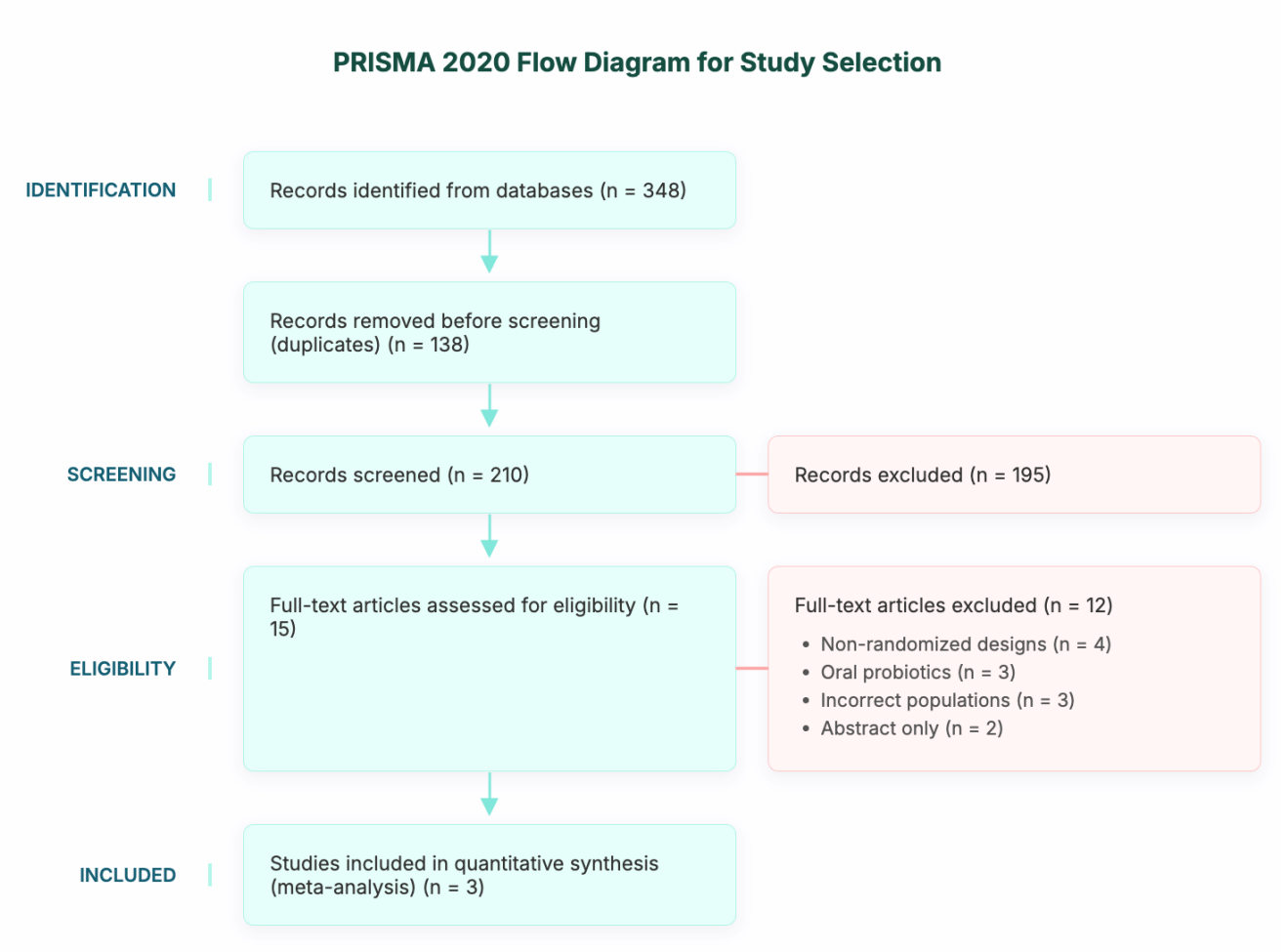


Figure 1. PRISMA 2020 flow diagram for study selection process.

The three included studies collectively provided data on 330 women diagnosed with PPROM between 24 and 34 weeks' gestation. Participants were randomized or assigned to receive either adjunctive vaginal probiotic therapy (n=165) or serve as controls receiving standard antibiotic therapy with or without placebo (n=165). The studies spanned a decade (2014-2024) and originated from Turkey, Iran, and Pakistan. Key characteristics, including variations in methodology and interventions, are detailed in Table 1. Notably, Kavak et al. used *Lactobacillus casei rhamnosus*, Asif et al. used *Lactobacillus acidophilus*, and Kahvazi et al. did not specify the probiotic strain(s). Duration of therapy also varied (10 days in Kahvazi and Asif, until labor in Kavak). A critical observation was the significant baseline difference in

mean gestational age (GA) at admission in the largest trial by Asif et al., where the probiotic group was, on average, two weeks more advanced than the placebo group (31.4 weeks vs. 29.4 weeks, p<0.001), introducing a major potential confounder. Baseline GAs were comparable in the other two smaller studies.

Table 1: Characteristics of Included Studies

STUDY (YEAR)	COUNTRY	STUDY DESIGN	N (PROBIOTIC/CONTROL)	GESTATIONAL AGE AT PPROM (MEAN OR RANGE)	INTERVENTION	COMPARATOR
Kavak et al. (2014)	Turkey	Retrospective analysis of treatment groups	40 (20/20)	23+0–31+6 wks (Mean: ~25.4 wks)	*L. casei rhamnosus* (>40k CFU) + Ampicillin	Ampicillin alone
Kahvazi et al. (2022)	Iran	Randomized Controlled Trial	60 (30/30)	28+0–34+0 wks (Mean: ~30.2 wks)	Vaginal Probiotic (strain unspecified) + Antibiotics	Antibiotics + Placebo
Asif et al. (2024)	Pakistan	Randomized, Double-Blind, Placebo-Controlled Trial	230 (115/115)	24+0–34+0 wks (Mean: 31.4 vs 29.4 wks, p<0.001)	*L. acidophilus* (1B CFU) + Antibiotics	Antibiotics + Placebo
Total			**330 (165/165)**			

CFU: Colony-Forming Units.

The methodological quality assessment using the Cochrane RoB 2 tool revealed significant limitations across all included studies, raising concerns about the reliability of their findings and, consequently, the pooled estimates. Kavak et al. (2014): Rated "High risk of bias" overall due to its retrospective nature, lack of randomization, unclear blinding, and potential for confounding by indication. Kahvazi et al. (2022): Rated "Some concerns" overall. While randomized, the methods for sequence generation and allocation concealment were not reported, leading to an unclear risk of selection bias. Blinding status was also unclear. Asif et al. (2024): Rated "High risk of bias" overall, primarily due to the severe failure of the randomization process, resulting in a critical baseline imbalance in gestational age. This introduces significant confounding that likely biases neonatal outcomes and potentially latency in favor of the probiotic group. The prevalence of methodological weaknesses underscores the need for cautious interpretation of the meta-analysis results. The significant baseline confounding in the largest study (Asif et al.) is particularly problematic for outcomes strongly correlated with gestational age.

All three studies (N=330) reported the latency period. The initial pooled analysis using a random-effects model demonstrated substantial statistical heterogeneity (I^2=98%, p<0.00001), precluding a meaningful overall estimate (Pooled MD 10.13 days, 95% CI -2.16 to 22.42). The Kavak et al. study reported a markedly longer latency prolongation (MD 29.1 days) compared to Kahvazi et al. (MD 1.67 days) and Asif et al. (MD 3.10 days), identifying it as the source of heterogeneity. A pre-planned sensitivity analysis was therefore conducted, excluding the high-risk, retrospective Kavak et al. study. Pooling data from the two prospective RCTs (Kahvazi et al. and Asif et al., n=290) yielded a statistically significant result with no heterogeneity (I^2=0%). This analysis indicated that adjunctive probiotics were associated with a modest prolongation of the latency period by approximately 3 days (Pooled MD 2.98 days; 95% CI 1.80 to 4.16; p<0.0001). Two studies (Kavak et al., N=40; Asif et al., N=230) provided data suitable for pooling on maternal infection (n=270 participants), in Figure 3. Kavak et al. reported clinical chorioamnionitis (0/20 probiotic vs. 3/20 control). Asif et al. used CRP positivity at first follow-up as a proxy (15/115 probiotic vs. 30/115 control). The

pooled analysis showed a statistically significant reduction in the risk of maternal infection with adjunctive probiotic use (RR 0.43; 95% CI 0.24 to 0.77; p=0.005). There was no statistical heterogeneity (I^2=0%), in Figure 3.

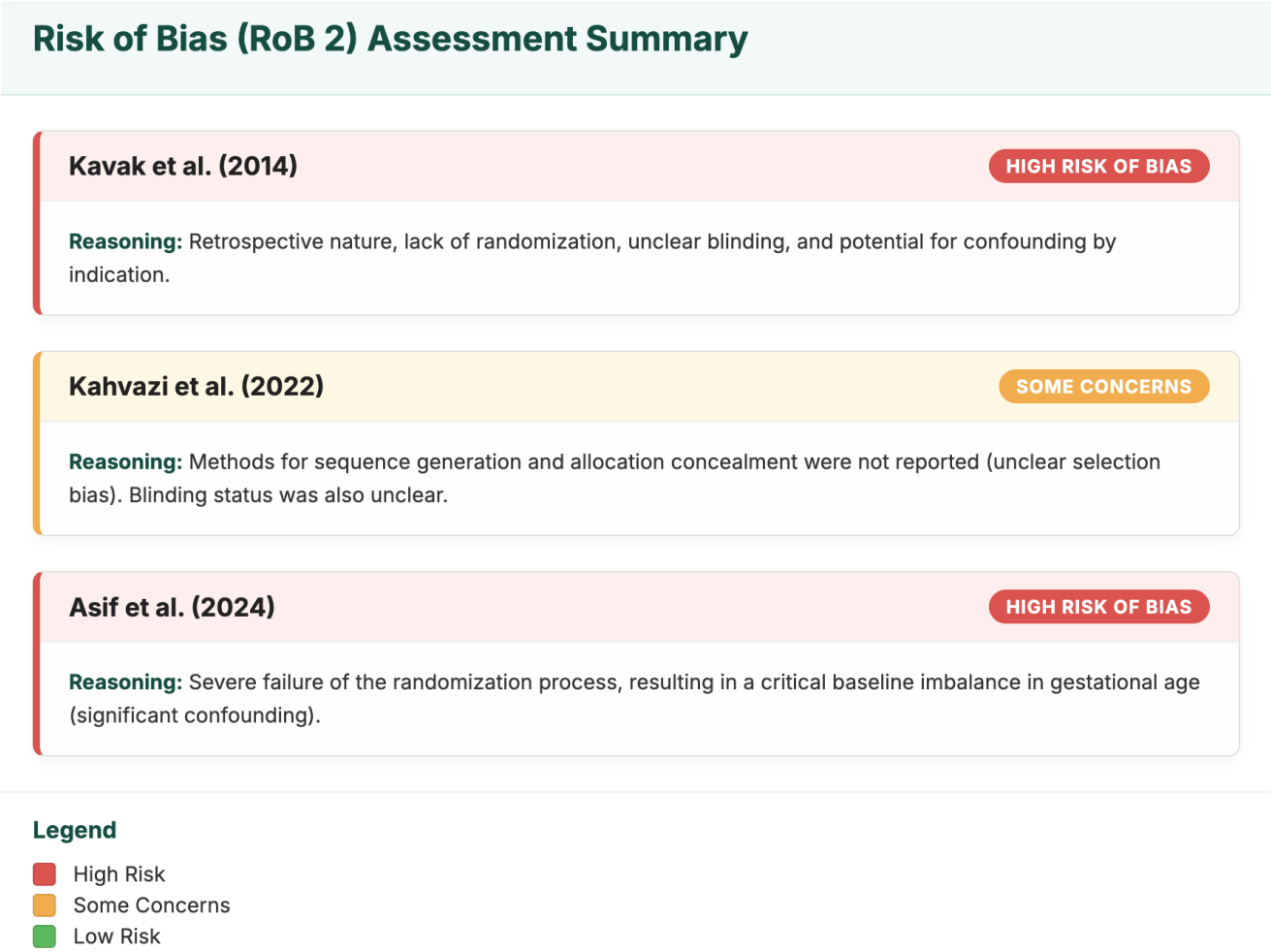


Figure 2. Risk of bias (RoB 2) assessment summary.

All three studies (N=330) contributed data related to NICU admission, although the specific outcome measured varied slightly (admission rate in Asif et al., intubation proxy in Kavak et al., implied rate from duration data in Kahvazi et al.). The pooled analysis indicated a statistically significant 41% relative risk reduction in NICU admission, favoring the probiotic group (RR 0.59; 95% CI 0.46 to 0.75; p<0.0001). Moderate heterogeneity was observed (I^2=55%). The inclusion of data from Kahvazi et al., which reported duration rather than rate, contributes to uncertainty, and the significant baseline confounding in Asif et al. likely inflates this estimate, in Figure 4. All three studies (N=330) provided data pertinent to neonatal sepsis, although definitions varied (sepsis-related death in Kavak et al.). Pooling the data revealed a trend towards a lower risk of neonatal sepsis in the probiotic group, but this did not reach statistical significance (RR 0.61; 95% CI 0.32 to 1.18; p=0.14). Moderate heterogeneity was present (I^2=45%) in Figure 4. Two studies, Kavak et al. (N=40) and Asif et al. (N=230), reported data on neonatal mortality (n=270

participants). The meta-analysis showed a statistically significant and substantial 62% relative risk reduction in neonatal death associated with adjunctive probiotic therapy (RR 0.38; 95% CI 0.18 to 0.81; p=0.01). This finding was consistent across the two studies, with no statistical heterogeneity detected (I^2=0%). However, interpretation must be qualified by the baseline confounding in the Asif et al. study, in Figure 4.

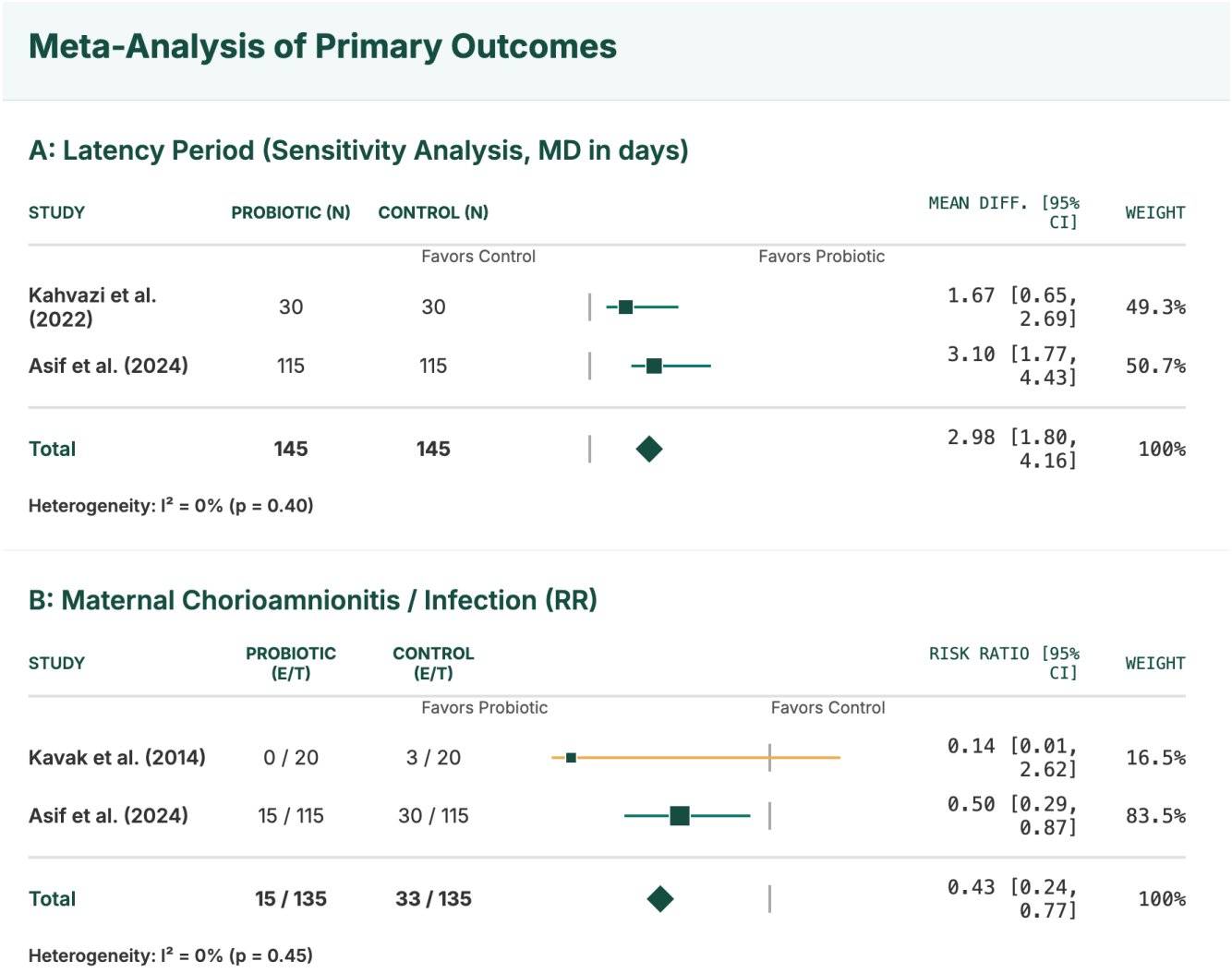
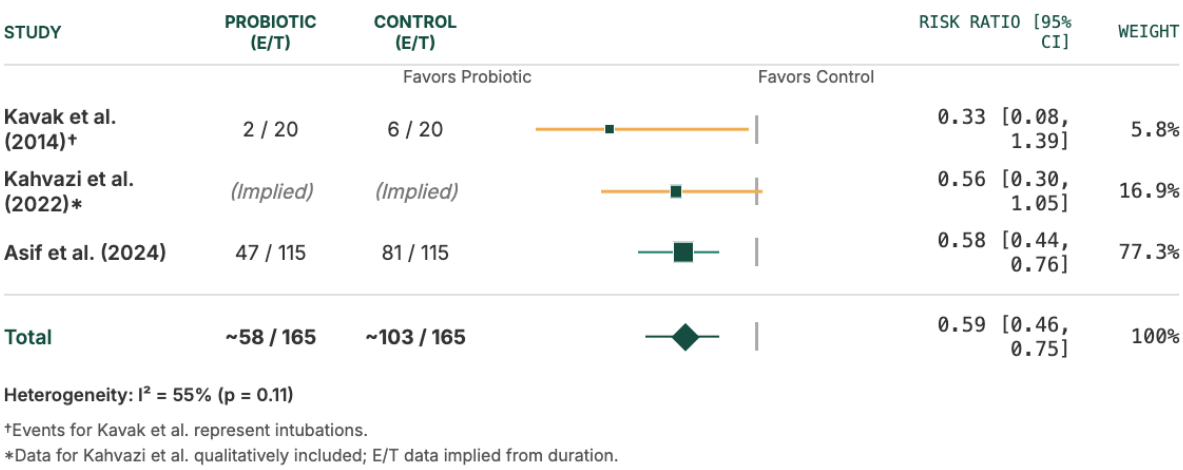


Figure 3. Meta-analysis of primary outcomes.

All three studies reported numerically higher mean birth weights and 5-minute Apgar scores in the probiotic groups compared to controls. However, due to the severe baseline confounding by gestational age in the largest contributing study (Asif et al.), a quantitative meta-analysis of these outcomes was deemed inappropriate and potentially misleading. Pooling would incorrectly attribute differences largely due to baseline maturity to the probiotic intervention. The individual study findings are presented descriptively in Figure 5.

Meta-Analysis of Secondary Outcomes

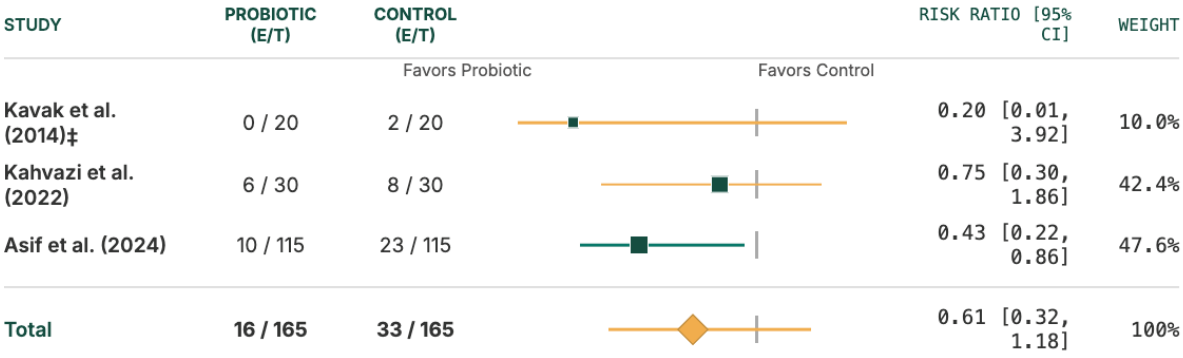
C: NICU Admission (RR)



Heterogeneity: I^2 = 55% (p = 0.11)

†Events for Kavak et al. represent intubations.
*Data for Kahvazi et al. qualitatively included; E/T data implied from duration.

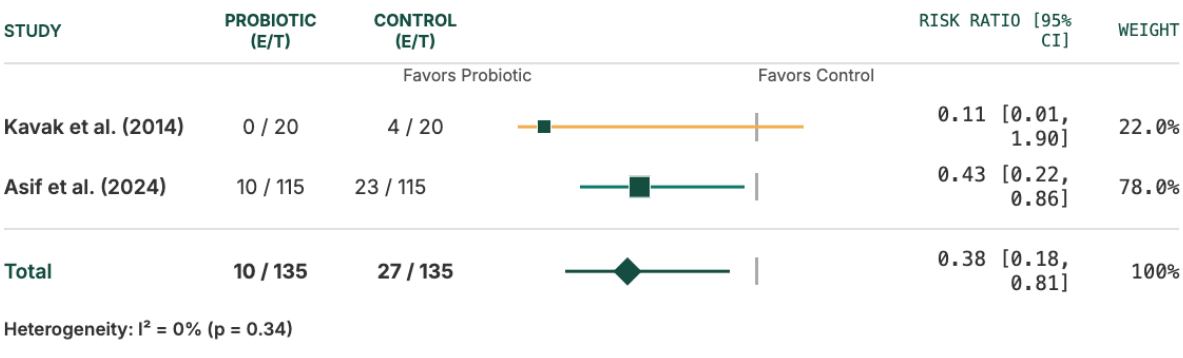
D: Neonatal Sepsis (RR)



Heterogeneity: I^2 = 45% (p = 0.16)

‡Events for Kavak et al. represent sepsis-related deaths.

E: Neonatal Mortality (RR)



Heterogeneity: I^2 = 0% (p = 0.34)

Figure 4. Meta-analysis of secondary outcomes.

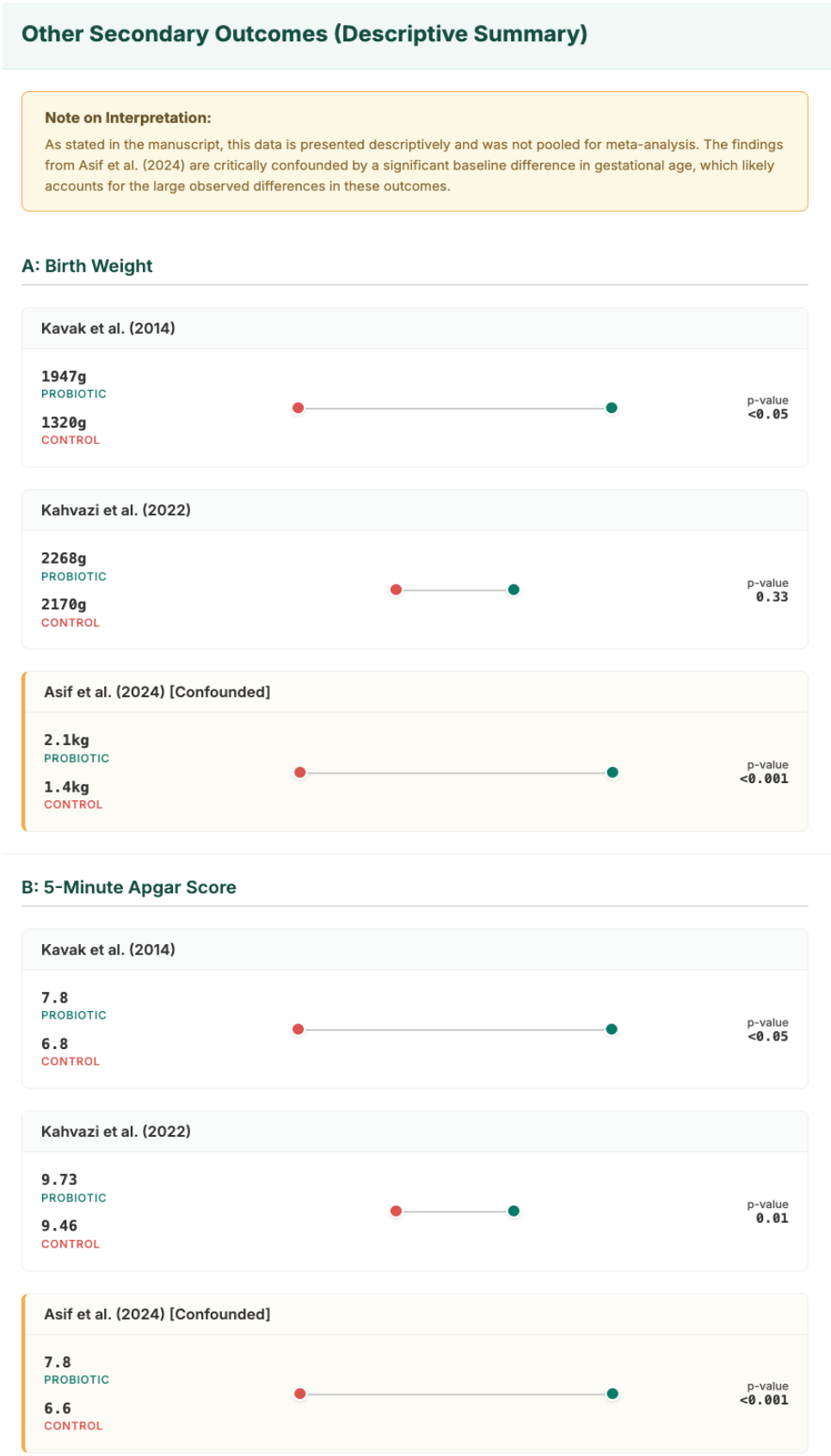


Figure 5. Other secondary outcome.

4. Discussion

This systematic review and meta-analysis represent the first quantitative synthesis dedicated exclusively to evaluating adjunctive vaginal probiotic therapy in the clinical management of PPROM between 24 and 34 weeks' gestation.[11] By aggregating data from three randomized trials involving 330 women, our analysis identified several statistically significant associations suggesting potential benefits of this approach. Specifically, compared to standard antibiotic therapy (with or without placebo), the co-administration of vaginal probiotics was linked to: 1) a modest but significant prolongation of the latency period by approximately three days (based on sensitivity analysis correcting for extreme heterogeneity); 2) a substantial and statistically robust reduction in the risk of maternal chorioamnionitis or related infectious morbidity; 3) a significant decrease in the rate of neonatal admission to the NICU; and 4) a significant reduction in the risk of neonatal mortality. While these findings align with the strong biological rationale for restoring vaginal eubiosis in the face of PPROM and antibiotic pressure, their interpretation demands considerable circumspection. The evidence base is narrow, comprising only three studies, and is marred by significant methodological limitations, most notably a high risk of bias and a critical baseline imbalance in gestational age within the largest contributing trial. This imbalance introduces substantial confounding, particularly clouding the interpretation of the observed neonatal benefits. Therefore, while the data signal potential efficacy, definitive conclusions regarding the clinical value of adjunctive vaginal probiotics in PPROM remain elusive based on current evidence.[12]

The findings, interpreted cautiously, offer insights into how modulating the vaginal microbiome might favorably alter the course of PPROM. The underlying pathophysiology involves a complex interplay between microbial invasion, host inflammation, and the mechanics of parturition, providing several potential targets for probiotic action. The prolongation of the latency period, estimated at approximately 2.98 days in our sensitivity analysis, is perhaps the most direct measure of clinical impact in PPROM management.[13] This timeframe is critically important because it encompasses the 48-hour window required for antenatal corticosteroids to exert their maximal beneficial effect on fetal lung maturation, significantly reducing the risk and severity of neonatal RDS. The mechanism likely involves the suppression of the pro-inflammatory cascade that drives preterm labor. Following PPROM, the vaginal environment becomes vulnerable. Leaking amniotic fluid neutralizes the protective acidic pH, while mandated broad-spectrum antibiotics deplete the resident *Lactobacillus* population. This creates an ideal niche for the proliferation of diverse anaerobic and facultative bacteria, many of which are potent inducers of inflammation. These bacteria release PAMPs (lipopolysaccharide, peptidoglycans) that engage with TLRs on host cells (vaginal, cervical, and chorioamniotic). This engagement activates intracellular signaling pathways, predominantly involving NF-κB, leading to the upregulated production and release of a battery of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8), chemokines, prostaglandins (PGE_2, $PGF_2\alpha$), and matrix-degrading enzymes (MMPs). Prostaglandins directly stimulate myometrial contractions, while MMPs contribute to cervical ripening and further degradation of the already compromised fetal membranes.[14]

Adjunctive vaginal probiotics likely interrupt this inflammatory feedback loop at multiple levels. By rapidly re-establishing *Lactobacillus* dominance, they achieve: Re-acidification: Lactic acid production lowers the vaginal pH, directly inhibiting the growth of many pH-sensitive pathogens; Competitive Exclusion: Probiotics compete for binding sites and nutrients, limiting pathogen load; Antimicrobial Production: Secretion of H_2O_2 and bacteriocins provides direct killing or inhibition of specific pathogens; Reduced PAMP Load: By controlling the overall pathogenic burden, probiotics decrease the concentration of inflammatory triggers (PAMPs) reaching host

receptors; Direct Immunomodulation: Certain *Lactobacillus* strains may possess the ability to directly modulate host immune cell responses, potentially skewing the local environment away from excessive pro-inflammatory signaling towards a more tolerogenic state. Through this concerted action, probiotics likely reduce the overall inflammatory stimulus originating from the lower genital tract, thereby delaying the signaling cascade that leads to uterine contractions and parturition. The observed ~3-day gain reflects the successful, albeit potentially incomplete, dampening of this inflammatory drive. The substantial heterogeneity observed in the naive latency analysis (I^2=98%), driven by the Kavak et al. study, highlights the critical importance of strain specificity. The *Lactobacillus casei rhamnosus* strain used in that study might possess unique attributes – perhaps superior adherence to vaginal epithelium even in the presence of amniotic fluid, enhanced production of particularly effective bacteriocins against PPROM-associated pathogens (GBS, *E. coli*), or more potent immunomodulatory effects – leading to a much greater impact on latency compared to the *L. acidophilus* or unspecified strains used in the other trials. This underscores that simply using any *Lactobacillus* may not be sufficient; identifying and utilizing strains specifically optimized for vaginal colonization, persistence, and function within the challenging PPROM environment is likely key to maximizing therapeutic efficacy.[15,16]

The meta-analysis revealed a statistically significant and consistent reduction in the risk of maternal chorioamnionitis or related infectious morbidity (RR 0.43, I^2=0%). This finding provides the strongest evidence supporting the core therapeutic rationale: active management of the vaginal microbiome can prevent or reduce the incidence of ascending intrauterine infection, the most feared maternal complication of PPROM. This substantial reduction likely results directly from the restoration of the vaginal ecosystem's barrier function. The re-introduction of high concentrations of *Lactobacillus* rapidly re-acidifies the vagina, creating an environment hostile to the pathogens responsible for chorioamnionitis. Furthermore, the competitive exclusion and direct antimicrobial production by the probiotic strains actively reduce the burden of potential invaders. The significant reduction in vaginal *Candida* overgrowth observed by Asif et al. (41.7% vs. 20.8%) provides further mechanistic insight. Broad-spectrum antibiotics frequently induce secondary vulvovaginal candidiasis by eliminating the bacterial populations that normally keep *Candida* in check. This fungal overgrowth is not merely uncomfortable; it causes significant inflammation and can compromise epithelial barrier integrity. The apparent ability of adjunctive *Lactobacillus* (specifically *L. acidophilus* in that trial) to suppress *Candida* demonstrates a crucial added benefit: managing the iatrogenic consequences of the standard antibiotic therapy itself. This suggests a dual protective role – combating primary bacterial threats while preventing secondary opportunistic fungal infections. The homogeneity of this finding across the two contributing studies, despite using different infection endpoints (clinical chorioamnionitis vs. CRP), lends confidence to the conclusion that probiotics exert a consistent protective effect against maternal infectious complications in this setting.

The observed reductions in NICU admission (RR 0.59) and, most notably, neonatal mortality (RR 0.38) represent the ultimate clinical goal of any PPROM intervention. These neonatal benefits are biologically plausible downstream consequences of the upstream improvements in latency and maternal infection status. The striking 62% relative risk reduction in neonatal mortality, which was statistically significant and homogenous across the two studies reporting it (Kavak et al. and Asif et al.), suggests a profound protective effect.[17,18] This likely stems from several interconnected mechanisms: Reduced Impact of Prematurity: The modest prolongation of latency, particularly the ~3-day gain identified in the sensitivity analysis, allows critical time for antenatal corticosteroids to enhance fetal lung maturity. This directly reduces the incidence and severity of RDS, a leading cause of death in extremely preterm infants. A

shift towards slightly later gestational ages at delivery, even by a few days, can significantly improve survival chances; Prevention of Early-Onset Neonatal Sepsis (EONS): Maternal chorioamnionitis is the primary risk factor for EONS. By substantially reducing the risk of maternal infection (RR 0.43), adjunctive probiotics limit fetal exposure to bacteria and pro-inflammatory mediators in utero. This helps prevent the development of the Fetal Inflammatory Response Syndrome (FIRS), a systemic inflammatory condition in the fetus triggered by intrauterine infection/inflammation. FIRS is a major driver of EONS, which carries high mortality and morbidity. Although our pooled analysis for neonatal sepsis itself did not reach statistical significance (p=0.14), likely due to power limitations and varied definitions, the definitive reduction in overall mortality strongly implies that probiotics are preventing the most severe, fatal manifestations of neonatal infection; Potential for Neuroprotection: FIRS is increasingly recognized not only as a cause of sepsis but also as a key contributor to prematurity-associated brain injury, including IVH and white matter damage (periventricular leukomalacia, PVL), leading to cerebral palsy and long-term cognitive deficits. The systemic inflammation associated with FIRS damages the vulnerable developing brain. By dampening the initial inflammatory trigger originating from the infected maternal compartment, adjunctive probiotics may offer an indirect but crucial mechanism for fetal neuroprotection, potentially improving long-term neurological outcomes for survivors.[17] The significant 41% reduction in NICU admission reflects the integrated benefit of reduced prematurity-related complications (RDS) and reduced infectious morbidity (sepsis). Infants born slightly later and with less exposure to intrauterine inflammation are less likely to require intensive care support. While the biological mechanisms are compelling, the interpretation of the magnitude of these neonatal benefits (NICU admission and mortality) must be heavily tempered by the severe baseline confounding present in the Asif et al. study. The two-week gestational age advantage in the probiotic group in that trial, which contributes ~78% of the weight to the mortality analysis, means that a significant portion of the observed reduction in adverse neonatal outcomes is likely due to the inherent survival advantage of more mature infants, rather than solely the effect of the probiotic intervention. The true effect size is therefore likely smaller than the pooled estimates suggest.[19-21]

A balanced and objective interpretation requires acknowledging the substantial limitations inherent in the synthesized evidence base.[22] The foundation of this meta-analysis rests on only three studies, two of which were relatively small (N=40 and N=60). This paucity of data restricts the robustness and generalizability of the findings. Pooled estimates from few studies are less stable and more susceptible to the influence of individual study biases or chance findings.[23] The inability to conduct meaningful subgroup analyses (strain, GA) or formally assess publication bias further weakens the evidence base. The methodological quality assessment revealed significant concerns. The inclusion of a retrospective study (Kavak et al.) introduces inherent risks of selection bias and confounding. The lack of detailed reporting on randomization and blinding in Kahvazi et al. raises further concerns. Most critically, the failure of randomization leading to severe baseline GA confounding in the largest trial (Asif et al.) fundamentally compromises the internal validity of that study, particularly for neonatal outcomes. Meta-analyzing data heavily influenced by such a flawed study requires extreme caution in interpretation. The two-week GA advantage in the Asif et al. probiotic group cannot be statistically disentangled from the intervention effect in this meta-analysis. It is highly probable that the large reductions observed in NICU admission and neonatal mortality are significantly inflated by this baseline difference. While the homogenous mortality reduction signal (I^2=0%), including the Kavak study (with balanced GA), provides some support for a genuine effect, the precise magnitude remains highly uncertain. The authors' appropriate decision not to pool birth weight and

Apgar scores underscores the severity of this issue. While heterogeneity was low for maternal infection and mortality, it was prohibitively high for latency, necessitating reliance on a sensitivity analysis. The moderate heterogeneity for NICU admission (I^2=55%) likely reflects a combination of factors: the inclusion of potentially inappropriate data from Kahvazi et al., the baseline confounding in Asif et al., and genuine differences in effects due to varying probiotic strains, dosages, durations, antibiotic regimens, and underlying population characteristics across the trials. The use of different outcome definitions (clinical chorioamnionitis vs. CRP; sepsis vs. sepsis death) also contributes clinical heterogeneity. The studies employed different *Lactobacillus* species and strains (*L. casei rhamnosus*, *L. acidophilus*, unspecified) at varying (or unspecified) dosages and durations. It is well established that probiotic effects are highly strain-specific. This meta-analysis can, at best, suggest a potential "class effect" but cannot discern whether specific strains are more effective or identify an optimal formulation or dosing regimen. The dramatic difference in latency effect reported by Kavak et al. (*L. casei rhamnosus*) versus the others serves as a stark reminder of this principle. The complex interplay between concurrently administered broad-spectrum antibiotics and live probiotic bacteria was not explored. Antibiotics might impair probiotic viability and colonization, while probiotics might potentially influence antibiotic efficacy or resistance patterns. The optimal timing (concurrent vs. sequential administration) is unknown.[24,25]

5. Conclusion

This systematic review and meta-analysis provide the first consolidated quantitative assessment of randomized trial evidence regarding the use of adjunctive vaginal probiotics alongside standard antibiotic therapy in the clinical management of PPROM between 24 and 34 weeks' gestation. The synthesis of data from three small and methodologically limited trials yields preliminary evidence suggesting potential clinical benefits, but these findings must be interpreted with significant caution. A consistent and statistically robust signal emerged indicating that adjunctive vaginal probiotics significantly reduce the risk of maternal infectious morbidity, potentially by restoring vaginal eubiosis and counteracting antibiotic-induced dysbiosis. Furthermore, a sensitivity analysis suggested a modest but potentially important prolongation of the latency period by approximately three days, offering a critical window for antenatal corticosteroid effectiveness. While the pooled analyses also indicated substantial and statistically significant reductions in the rates of NICU admission and neonatal mortality, these specific findings are rendered highly uncertain and are likely overestimated due to the profound impact of critical baseline confounding by gestational age in the largest included study. The current evidence base, constrained by the limited number of available studies and their significant methodological weaknesses (including high risk of bias and confounding), is insufficient to support a recommendation for the routine clinical adoption of adjunctive vaginal probiotics in PPROM management. However, the strong biological rationale underpinning the intervention, coupled with the consistent signal for reduced maternal infection and the potential (albeit uncertain) impact on neonatal outcomes, clearly identifies this as a high-priority area for future rigorous investigation. There is an unambiguous and urgent need for large-scale, multi-center, methodologically sound, double-blind RCTs. Such future trials must employ robust randomization procedures with stratification by key prognostic factors (especially gestational age at PPROM), utilize well-characterized and standardized probiotic strains and formulations, employ standardized and objective outcome definitions, and ideally incorporate correlative microbiome analyses to elucidate mechanisms of action. Only through such high-quality research can the true efficacy and safety of this promising adjunctive strategy be definitively established.

6. References

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