

# The effect of interventions targeting gut microbiota on depressive symptoms: a systematic review and meta-analysis

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## Abstract

**Background:** Despite their popularity, the efficacy of interventions targeting gut microbiota to improve depressive symptoms is unknown. Our objective is to summarize the effect of microbiome-targeting interventions on depressive symptoms.

**Methods:** We conducted a systematic review and meta-analysis. We searched MEDLINE, Embase, PsycINFO, Database of Abstracts of Reviews of Effects, Cochrane Database of Systematic Reviews and the Cochrane Controlled Register of Trials from inception to Mar. 5, 2021. We included studies that evaluated probiotic, prebiotic, synbiotic, paraprobiotic or fecal microbiota transplant interventions in an adult population (age  $\geq 18$  yr) with an inactive or placebo comparator (defined by the absence of active intervention). Studies must have measured depressive symptoms with a validated scale, and used a randomized controlled trial study design. We conducted a random effects meta-analysis of change scores, using standardized mean difference as the measure of effect.

**Results:** Sixty-two studies formed the final data set, with 50 included in the meta-analysis. Probiotic, prebiotic, and synbiotic interventions on depressive symptoms showed statistically significant benefits. In the single studies evaluating each of fecal microbiota transplant and paraprobiotic interventions, neither showed a statistically significant benefit.

**Interpretation:** Despite promising findings of benefit of probiotic, prebiotic and synbiotic interventions for depressive symptoms in study populations, there is not yet strong enough evidence to favour inclusion of these interventions in treatment guidelines for depression. Critical questions about species administered, dosage and timing relative to other antidepressant medications remain to be answered. **Study registration:** PROSPERO no. 143178

Mounting evidence supports the concept of a microbiota–gut–brain axis and suggests that this axis is perturbed in neuropsychiatric disorders. The gut microbiota regulates host exposure to its products by modulating gut epithelial and blood–brain permeability,<sup>1,2</sup> both of which are altered in patients with major depressive disorder.<sup>3–6</sup> In addition, patients with major depressive disorder have shown substantial shifts in both the relative abundance of taxa and the neuroactive metabolic potential of the gut microbiota, compared with healthy controls.<sup>7–12</sup>

Because of this compelling preclinical data,<sup>1–12</sup> interventions affecting the microbiota–gut–brain axis are a potential treatment modality for depressive symptoms. Multiple systematic reviews have been conducted to assess the effect of microbiota-targeting interventions on depressive symptoms, but they include diverse populations and different study designs, include different subsets of the interventions targeting the gut microbiota and, not surprisingly, report conflicting findings.<sup>13–15</sup> The objective of this study is to summarize the effect of microbiome-targeting interventions on depressive symptoms.

## Methods

### Design

We conducted a systematic review and meta-analysis, following Cochrane recommendations for best practice, and the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) for reporting.<sup>16,17</sup> We registered the protocol with PROSPERO (ID: 143178). As domain knowledge was refined, so too was the protocol and analysis strategy, in consultation with domain experts; deviations from the registered protocol are outlined in Appendix 1, Section 1, available at [www.cmajopen.ca/content/9/4/E1195/suppl/DC1](http://www.cmajopen.ca/content/9/4/E1195/suppl/DC1). Briefly, we decided to focus on depressive symptoms, rather than all mental health outcomes, to enhance interpretability.

**Competing interests:** None declared.

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### Search strategy

On July 3, 2019, we searched MEDLINE, Embase, PsycINFO, the Database of Abstracts of Reviews of Effects, Cochrane Database of Systematic Reviews and the Cochrane Controlled Register of Trials from inception; we updated our search on Mar. 5, 2021. We used search terms for gut microbiota-targeting interventions and depression, such as “probiotics” and “depression.” Search terms were intentionally broad, to avoid excluding relevant interventions or outcomes at this stage. We searched Medical Subject Headings (MeSH), text words and keywords (Appendix 1, Section 2). A research librarian developed the search strategy, which underwent Peer Review of Electronic Search Strategies (PRESS) review.<sup>18</sup>

We filtered search results to exclude studies published in a language other than English or French, those using animal models, and commentaries, editorials, letters and case reports. One author hand-searched reference lists of identified systematic reviews.

### Study selection

Eight authors (M.H., J.L., L.E.D., B.F., L.M., O.E., R.D., N.C.A.C.) screened titles and abstracts independently and in duplicate. During title and abstract screening, we refined inclusion criteria in consultation with domain experts. To ensure that all abstract reviewers shared an understanding of the review objective, reviewers and domain experts calibrated with batches of 100 abstracts until 100% agreement was reached, before proceeding to review all remaining abstracts independently and in duplicate. We used the same procedure at full-text assessment, with batches of 10 full-texts assessed by the same 8 authors (M.H., J.L., L.E.D., B.F., L.M., O.E., R.D., N.C.A.C.). Any citation included by either reviewer proceeded to full-text review, which was also conducted independently and in duplicate. Reviewers discussed disagreements until consensus was reached.

We included randomized controlled trials that evaluated microbiome-targeting interventions (i.e., probiotic, prebiotic, synbiotic, paraprobiotic or fecal microbiota transplant) in adults aged 18 and older, that measured depressive symptoms with a validated scale and used a placebo or control comparator in which the active substance in the intervention was not administered (Table 1). We considered any study population for inclusion. To be considered a validated outcome, we required there to be a publication describing validity of each tool in any population (Appendix 1, Section 3).

### Data extraction and quality assessment

In addition to assessing study quality, 8 authors (M.H., J.L., L.E.D., B.F., L.M., O.E., R.D., N.C.A.C.) used standardized forms to extract author, year, study design, population inclusion and exclusion criteria, follow-up, sample size, intervention(s), dose, additional supplements, depressive symptom outcome(s), independently and in duplicate. They also assessed study quality with the Cochrane Risk of Bias 2.0 tool.<sup>19</sup> We used a hierarchy developed by an expert psychiatrist a priori to select an outcome from each study for inclusion in meta-analysis<sup>20</sup> when the same mental health

**Table 1: Study inclusion criteria**

Criterion	Description
Population	Human, aged 18 years or older
Intervention	Probiotic (consumption of live microorganisms) Prebiotic (compound[s] to induce growth or activity of gut microbiota) Synbiotic (combination of probiotic and prebiotic) Paraprobiotic (sterilized or inactivated bacteria) Fecal microbiota transplant
Comparator	Placebo or control, defined by the absence of intervention
Outcome	Depressive symptoms, measured with a validated tool
Design	Randomized controlled trials

outcome was measured with more than 1 validated tool, whereby we prioritized observer-rated tools above self-rated tools, commonly used tools over less commonly used tools, and tools measuring specific symptoms over those measuring mixed symptoms.

### Statistical analysis

We used random effects models with methods described by DerSimonian and Laird,<sup>21</sup> as specified for meta-analysis a priori. We summarized effect size with the standardized mean difference of change scores after treatment, which expresses difference in effects between interventions in units of standard deviations. In accordance with the Cochrane Collaboration’s recommendations for best practice, we used Hedges’ *g* to correct for bias, often encountered in studies of small sample size.<sup>22</sup> Where only pre- and post-treatment scores were provided, we used the conservative correlation coefficient of 0.5 to estimate change scores.<sup>23</sup> We summarized heterogeneity quantitatively with *I*<sup>2</sup>.

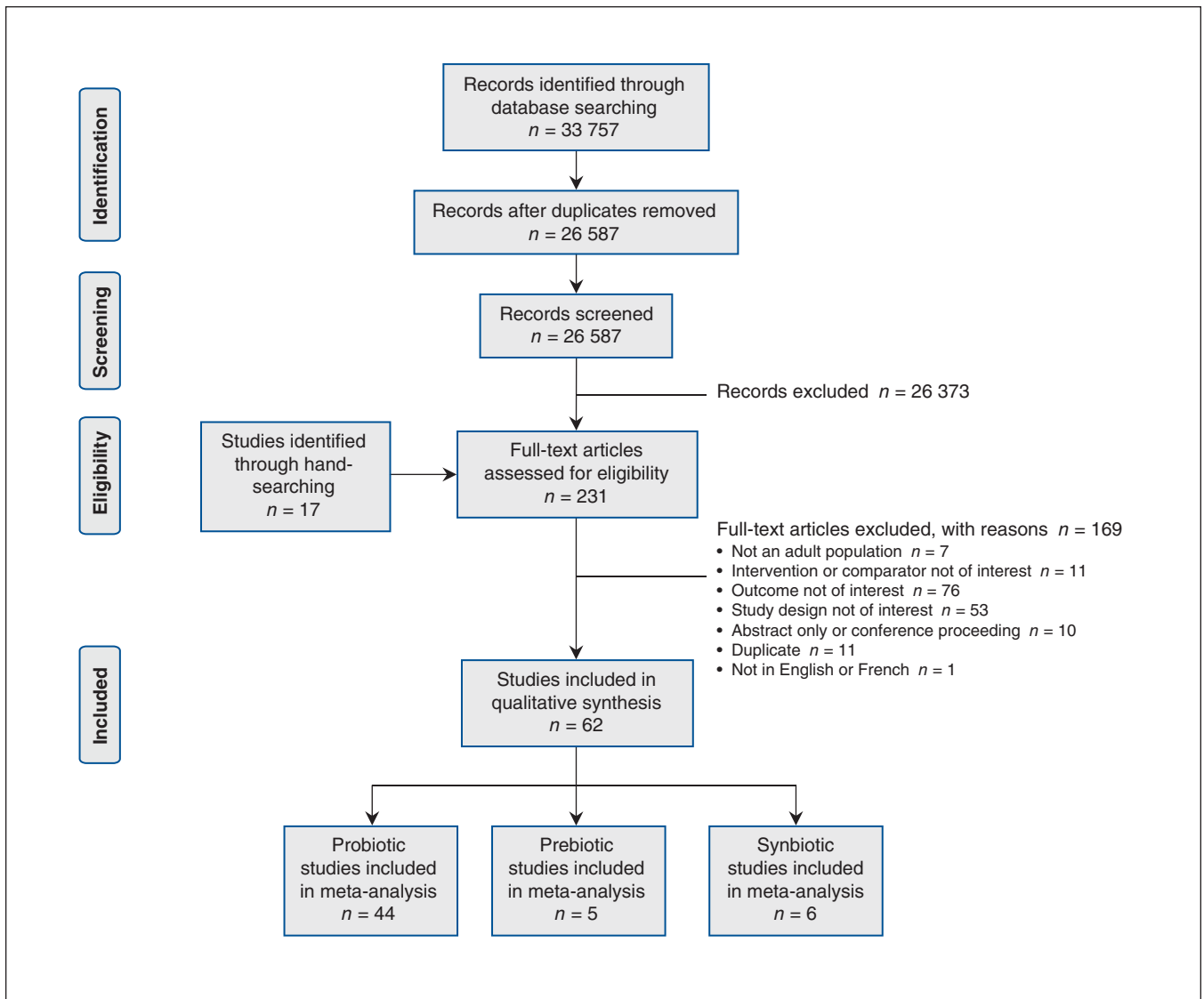
We conducted the meta-analysis and generated forest plots with the “metafor” package for R statistical software, and generated figures with the “ggplot2” package. We considered participant populations with a diagnosis of depression at baseline separately from participant populations where the presence of depression at baseline was not specified. In a sensitivity analysis, we removed studies deemed high risk of bias from estimates of effect. We visually inspected funnel plots for publication bias, and supplemented with trim and fill analysis.<sup>24</sup>

### Ethics approval

Because this analysis uses only previously published data, ethics approval was not required.

### Results

We identified 33 757 unique records. After abstract review, we assessed 231 full texts for eligibility, including 17 records identified through hand-searching. Of the full texts, we excluded



**Figure 1:** Flow diagram, based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist.<sup>16</sup>

169 for the following reasons: not adult population ( $n = 7$ ), intervention or comparator not of interest ( $n = 11$ ), outcome not of interest ( $n = 76$ ), study design not of interest ( $n = 53$ ), abstract only or conference proceeding ( $n = 10$ ), duplicate ( $n = 11$ ) and not available in English or French ( $n = 1$ ) (Figure 1). Reasons for full text exclusion are in Appendix 1, Section 4. The final data set included 62 studies with 5059 patients.

### Study characteristics

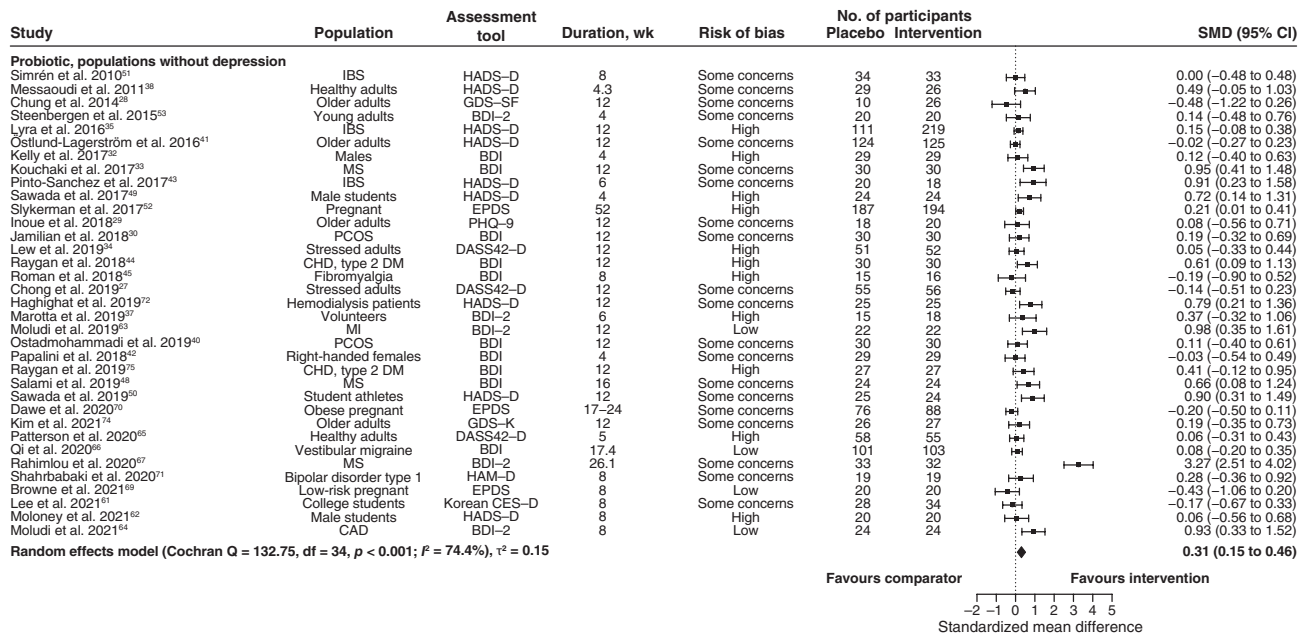
Characteristics of included studies can be found in Appendix 1, Section 5. The most common intervention type was probiotics ( $n = 51$ ),<sup>25-75</sup> followed by synbiotics ( $n = 7$ ),<sup>64,72,76-80</sup> prebiotics ( $n = 7$ ),<sup>31,64,73,81-84</sup> paraprobiotics ( $n = 1$ )<sup>85</sup> and fecal microbiota transplant ( $n = 1$  study).<sup>86</sup> Four studies included more than 1 active intervention, with each intervention included separately in our meta-analysis.<sup>31,64,72,73</sup> Sixteen distinct tools were used to evaluate depressive symptoms. The

most used tools were the Hospital Anxiety and Depression Scale — Depression score ( $n = 18$ ) and the Beck Depression Inventory ( $n = 16$ ).

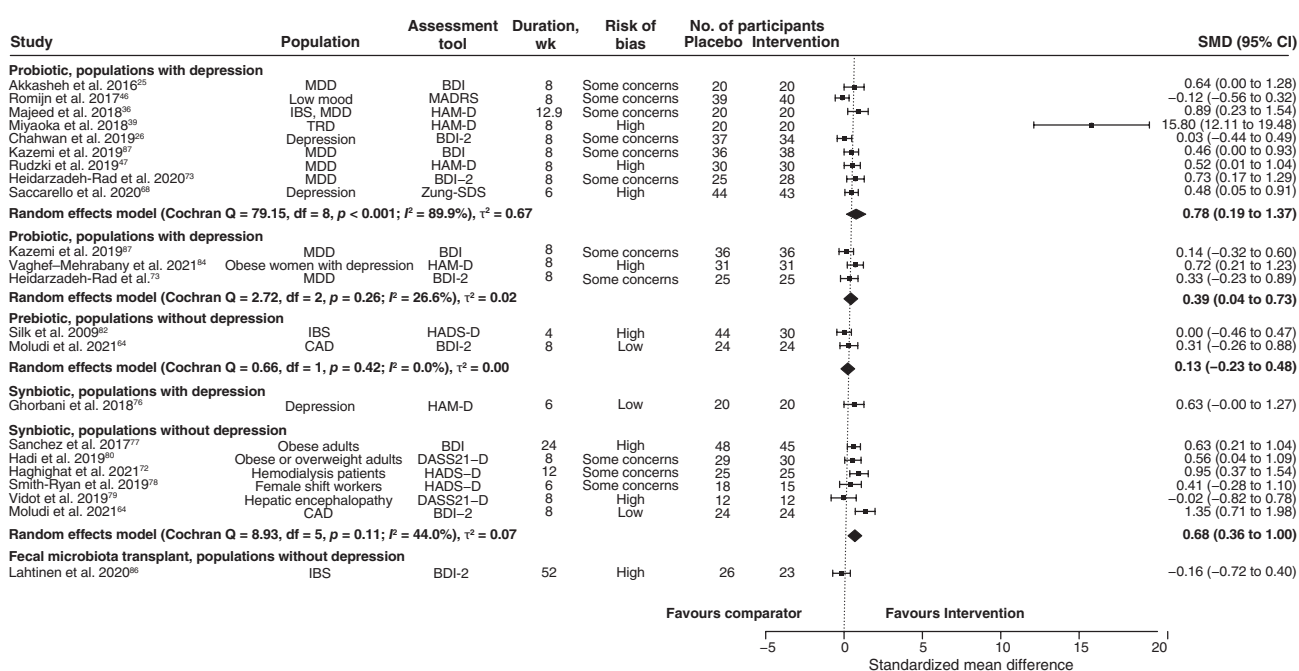
We excluded 2 studies from the meta-analysis, given the lack of studies with the same intervention or population with which to pool effect sizes.<sup>76,86</sup> Of the 50 studies included in the meta-analysis, the intervention was a probiotic in 44 studies ( $n = 9$  in populations with depression,  $n = 35$  in populations without depression), a prebiotic in 5 studies ( $n = 3$  in populations with depression,  $n = 2$  in populations without depression) and a synbiotic in 6 studies (all in populations without depression).

One study evaluated synbiotics in a population with depression,<sup>76</sup> and another evaluated fecal microbiota transplant in a population without depression;<sup>86</sup> neither of these had other studies with which to pool effect estimates. These 2 studies presented sufficient information for meta-analysis and are therefore included in Figure 2.

**A**



**B**



**Figure 2:** Forest plot of (A) probiotic interventions in populations without depression, (B) probiotic interventions in populations with depression, prebiotic interventions in populations with and without depression, synbiotic interventions in populations with and without depression, and fecal microbiota transplant interventions in populations without depression. Note: BDI = Beck Depression Inventory, CAD = coronary artery disease, CES-D = Centre for Epidemiological Studies Depression Scale, CHD = coronary heart disease, CI = confidence interval, DASS21-D = Depression Anxiety and Stress Scales – 21 Items, Depression Scale, DASS42-D = Depression Anxiety Stress Scales – 42 Items, Depression Scale, DM = diabetes mellitus, EPDS = Edinburgh Postnatal Depression Scale, GDS-K = Geriatric Depression Scale – Korean Version, GDS-SF = Geriatric Depression Scale – Short Form, HADS-D = Hospital Anxiety and Depression Scale – Depression score, HAM-D = Hamilton Depression Rating Scale, IBS = irritable bowel syndrome, MADRS = Montgomery-Åsberg Depression Rating Scale, MDD = major depressive disorder, MI = myocardial infarction, MS = multiple sclerosis, PCOS = polycystic ovary syndrome, PHQ-9 = Patient Health Questionnaire – 9, SMD = standardized mean difference, TRD = treatment-resistant depression, Zung-SDS = Zung Self-Rating Depression Scale.

The remaining 10 studies failed to present necessary information for inclusion in meta-analysis. Of these 10 studies, 7 studies evaluated a probiotic, 2 studies evaluated a prebiotic and 1 study evaluated a paraprobiotic (Appendix 1, Section 6). None of the studies that had insufficient information for meta-analysis reported statistically significant differences from interventions.

**Probiotic interventions**

Among studies with probiotic interventions, defined as consumption of live microorganisms, the most common genera of bacteria administered were *Lactobacillus* (n = 41) and *Bifidobacterium* (n = 29). Other genera administered were *Bacillus*, *Clostridium*, *Lactococcus*, *Streptococcus*, *Weisella* and *Lacticaseibacillus*. Twenty-four studies administered probiotics from more than 1 genus. Among 9 studies with participants with depression, probiotic interventions offered statistically significant benefits (Hedges' g 0.78, 95% confidence interval [CI] 0.19 to 1.37,  $\tau^2 = 0.67$ ,  $I^2 = 89.9\%$ ) (Figure 2).

One study, a visual outlier in Figure 2, was unique in the administration of *Clostridium*.<sup>39</sup> This study by Miyaoka and colleagues<sup>39</sup> was also unique in the requirement that participants with treatment-resistant depression be on a stable dose of selective serotonin reuptake inhibitor or serotonin-noradrenalin reuptake inhibitor for at least 1 month before enrolment. Exclusion of the visual outlier resulted in an effect size of 0.41 (95% CI 0.17 to 0.65,  $\tau^2 = 0.05$ ,  $I^2 = 42.9\%$ ), with markedly reduced heterogeneity and between-study variance.

In 35 studies that enrolled participants without depression, probiotics also offered statistically significant benefits (Hedges' g 0.31, 95% CI 0.15 to 0.46,  $\tau^2 = 0.15$ ,  $I^2 = 74.4\%$ ) (Figure 2).

**Prebiotic interventions**

We identified 7 studies evaluating the effect of prebiotic interventions, or compounds in food that induce growth or activity of gut microbiota.<sup>64,73,81-84,87</sup> Three studies with prebiotic interventions enrolled participants with depression, with statistically significant benefits (Hedges' g 0.39, 95% CI 0.04 to 0.73,  $\tau^2 = 0.02$ ,  $I^2 = 26.6\%$ ) (Figure 2). Among 2 studies enrolling participants without depression, we did not observe any statistically significant effects (Hedges' g 0.13, 95% CI -0.23 to 0.48,  $\tau^2 = 0.00$ ,  $I^2 = 00.0\%$ ) (Figure 2).

**Synbiotic interventions**

Seven studies evaluated the effects of synbiotics, or combinations of prebiotics and probiotics.<sup>64,72,76-80</sup> In the meta-analysis of 6 study populations without depression, synbiotic interventions offered statistically significant benefit (Hedges' g 0.68, 95% CI 0.36 to 1.00,  $\tau^2 = 0.07$ ,  $I^2 = 44.0\%$ ). The seventh study,<sup>76</sup> conducted in participants with depression, did not find a significant effect (standardized mean difference 0.63, 95% CI -0.002 to 1.27) (Figure 2).

**Paraprobiotics**

One trial evaluated the effect of paraprobiotics, or sterilized bacteria, and reported no statistically significant effect of intervention when measured with the Hospital Anxiety and Depression Scale — Depression score.<sup>85</sup>

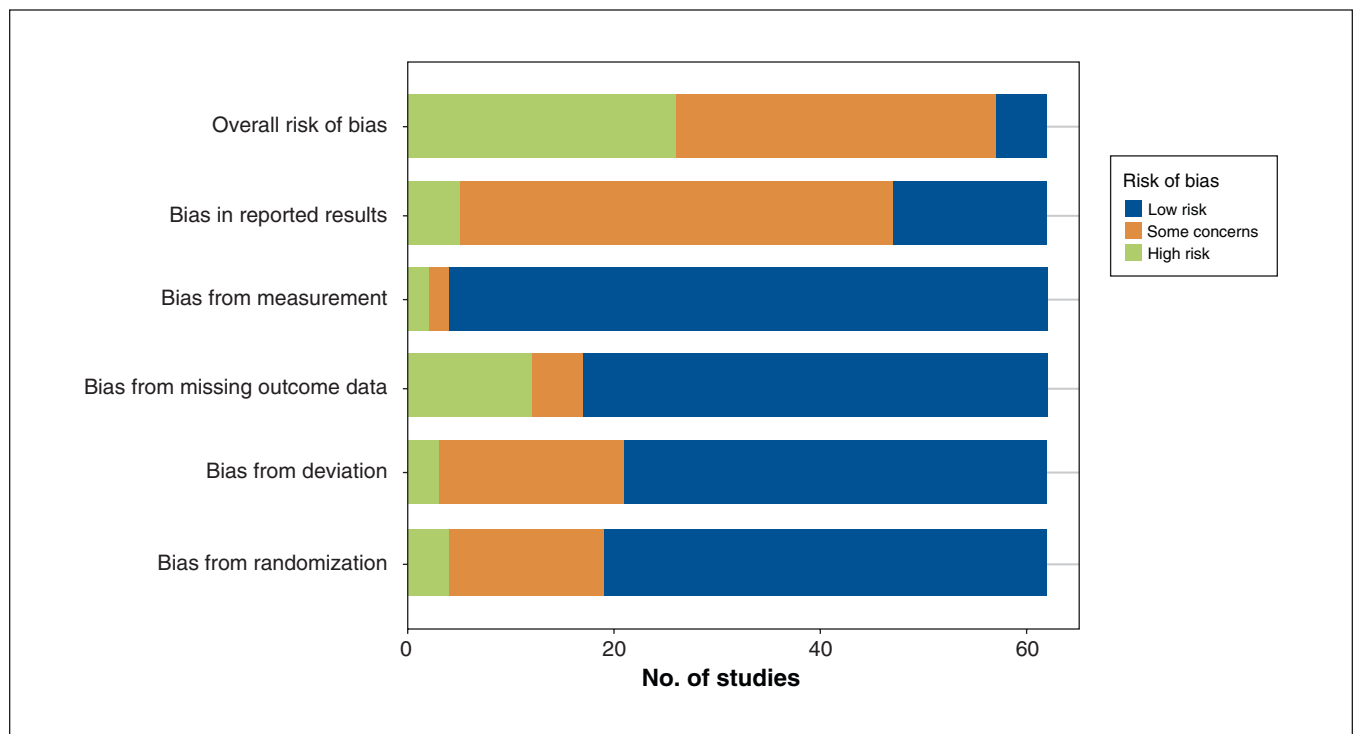


Figure 3: Risk of bias for included studies, assessed with the Cochrane Risk of Bias tool, version 2.0.<sup>19</sup>

### Fecal microbiota transplant

We identified 1 trial that evaluated the effect of fecal microbiota transplant.<sup>86</sup> In this study, patients with irritable bowel syndrome were randomized to autologous or allogenic fecal microbiota transplant via colonoscopy.<sup>86</sup> There were no differences in depressive symptoms, as measured with the Beck Depression Inventory, when compared with baseline or between groups at any time point (Figure 2).<sup>86</sup>

### Risk of bias

Although many studies were deemed low risk of bias in multiple domains, only 5 trials were deemed low risk of bias overall (Figure 3) (Appendix 1, Section 7).<sup>63,64,66,69,76</sup> Most studies were low risk of bias for their approach to measurement, but the study by Miyaoka and colleagues<sup>39</sup> was deemed high risk of bias in this domain given the lack of blinding.

### Sensitivity analysis

After removing studies deemed “high” risk of bias from meta-analysis, estimates of effect for probiotics in populations without depression and for prebiotics in populations with depression were similar to base case estimates. The magnitude of effect for probiotics in populations with depression was markedly smaller, with reduced between-study variance and heterogeneity. The magnitude of effect for synbiotics in populations without depression was larger than base case estimates, with reduced between-study variance and heterogeneity. Notably, we observed a statistically significant benefit in all analyses involving participants with depression (Table 2).

All 4 funnel plots show that few studies found intervention benefits with small standard error, suggesting the presence of publication bias (Figure 4). In our trim and fill analysis, excluding the study by Miyaoka and colleagues,<sup>39</sup> 2 missing studies are estimated on the left side of the funnel plot, with an effect estimate of 0.31 (95% CI 0.08–0.55,  $\tau^2 = 0.07$ ,  $I^2 = 50.4\%$ ) in

**Table 2: Summary of analyses**

Variable	Base case estimates	Estimates, excluding Miyaoka et al. <sup>39</sup>	Estimates, excluding studies deemed high risk of bias	Trim and fill analysis	Trim and fill analysis, excluding Miyaoka et al. <sup>39</sup>
<b>Probiotic interventions</b>					
Participants with depression	<b>9 studies</b> Hedges' <i>g</i> 0.78 (95% CI 0.19 to 1.37) $\tau^2 = 0.67$ $I^2 = 89.9\%$	<b>8 studies</b> Hedges' <i>g</i> 0.41 (95% CI 0.17 to 0.65) $\tau^2 = 0.05$ $I^2 = 42.9\%$	<b>6 studies</b> Hedges' <i>g</i> 0.39 (95% CI 0.07 to 0.72) $\tau^2 = 0.09$ $I^2 = 57.0\%$	<b>9 studies; 0 missing</b> Hedges' <i>g</i> 0.39 (95% CI 0.19 to 1.37) $\tau^2 = 0.67$ $I^2 = 89.9\%$	<b>9 studies; 2 missing</b> Hedges' <i>g</i> 0.31 (95% CI 0.08 to 0.55) $\tau^2 = 0.07$ $I^2 = 50.4\%$
Participants without depression	<b>35 studies</b> Hedges' <i>g</i> 0.31 (95% CI 0.15 to 0.46) $\tau^2 = 0.15$ $I^2 = 74.4\%$	NA	<b>24 studies</b> Hedges' <i>g</i> 0.36 (95% CI 0.13 to 0.59) $\tau^2 = 0.26$ $I^2 = 81.4\%$	<b>35 studies; 0 missing</b> Hedges' <i>g</i> 0.31 (95% CI 0.15 to 0.46) $\tau^2 = 0.15$ $I^2 = 74.4\%$	NA
<b>Prebiotic interventions</b>					
Participants with depression	<b>3 studies</b> Hedges' <i>g</i> 0.39 (95% CI 0.04 to 0.73) $\tau^2 = 0.02$ $I^2 = 26.6\%$	NA	<b>2 studies</b> Hedges' <i>g</i> 0.41 (95% CI 0.17 to 0.65) $\tau^2 = 0.05$ $I^2 = 42.9\%$	NA	NA
Participants without depression	<b>2 studies</b> Hedges' <i>g</i> 0.78 (95% CI 0.19 to 1.37) $\tau^2 = 0.67$ $I^2 = 89.9\%$	NA	NA	NA	NA
<b>Synbiotic interventions</b>					
Participants without depression	<b>6 studies</b> Hedges' <i>g</i> 0.68 (95% CI 0.36 to 1.00) $\tau^2 = 0.67$ $I^2 = 89.9\%$	NA	<b>4 studies</b> Hedges' <i>g</i> 0.82 (95% CI 0.42 to 1.21) $\tau^2 = 0.07$ $I^2 = 41.7\%$	<b>6 studies; 1 missing</b> Hedges' <i>g</i> 0.77 (95% CI 0.43 to 1.11) $\tau^2 = 0.11$ $I^2 = 54.1\%$	NA

Note: CI = confidence interval, NA = not applicable.

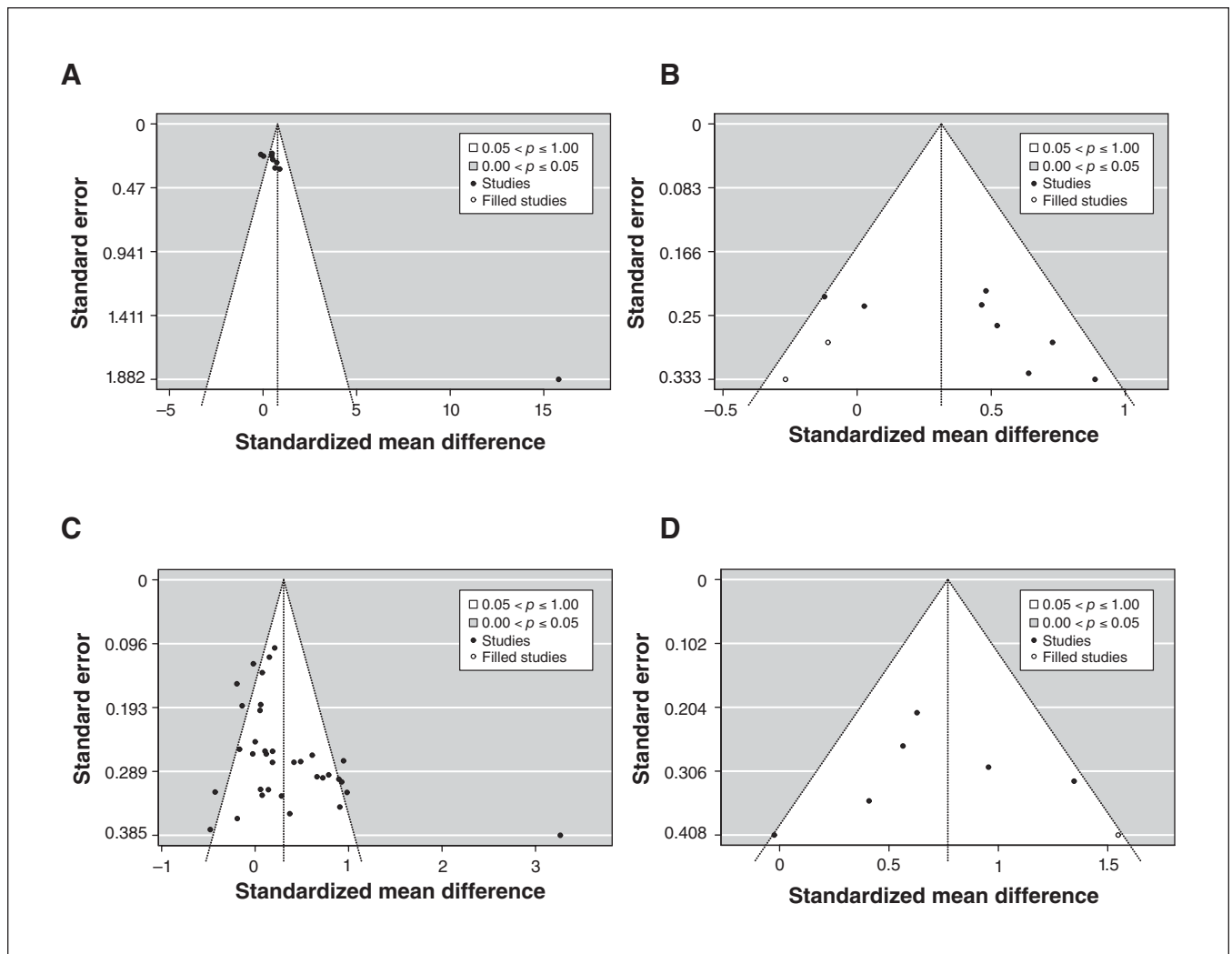
participants with depression (Figure 4B). In our trim and fill analysis of synbiotic interventions in populations without depression, 1 missing study is estimated on the right side of the funnel plot, with an effect estimate of 0.77 (95% CI 0.43 to 1.11,  $\tau^2 = 0.11$ ,  $I^2 = 54.1\%$ ). For other meta-analyses, there were insufficient studies to generate meaningful funnel plots.

### Interpretation

This meta-analysis suggests a statistically significant benefit of probiotic, prebiotic and synbiotic interventions for depressive symptoms in study populations, both with and without depression. None of the studies excluded from the meta-analysis for lack of required information showed statistically significant evidence of benefit. In the single studies evaluating fecal microbiota transplant and paraprobiotic interventions, the interventions did not show statistically significant benefits.<sup>85,86</sup> The body of evidence included in this systematic review is hindered by heterogeneous study quality and the likely presence of publication bias.

The lack of statistically significant evidence of benefit in many single studies may be from the measurement of depressive symptoms as a secondary outcome. Studies are rarely powered for measurement of secondary outcomes and, in the case of a small-to-medium effect size, they are underpowered to detect differences. If this is the case for studies examining paraprobiotic interventions or fecal microbiota transplants, further study and additional meta-analysis will be useful to improve precision in estimates of effect.

Effect sizes for synbiotic interventions were larger than for prebiotic or probiotic interventions, suggesting that the combination of interventions holds greater promise than solely prebiotic or probiotic interventions. Although complicated by risk of bias in included studies and the likely presence of publication bias, the magnitude of effect for synbiotic interventions in participants without depression is nearly the sum of prebiotic and probiotic interventions. Unfortunately, too few studies existed for meta-analysis of effects in participants with depression.



**Figure 4:** Funnel plots from trim and fill analysis of probiotic interventions in populations with depression, with (A) and without (B) study by Miyaoka et al.,<sup>39</sup> and of (C) probiotic and (D) synbiotic interventions in populations without depression.

The effect of the probiotic intervention reported by Miyaoka and colleagues<sup>39</sup> was an outlier. This was the only study administering adjunctive *Clostridium* to patients already being treated with antidepressant medications, for which change in depressive symptoms was a primary outcome. When this study was excluded, estimated effect sizes between groups with or without depression were of similar magnitude, with confidence intervals that overlap almost entirely. *Bifidobacterium*- and *Lactobacillus*-containing probiotics are produced commercially, are widely available and were used as the probiotic interventions in most included studies. The effect size estimated when excluding the study by Miyaoka and colleagues<sup>39</sup> may better reflect those achievable with commercially available products.

Although many studies evaluated effect sizes for similar species of bacteria, the 1 study that used *Clostridium* showed a far greater effect,<sup>39</sup> raising questions about why the body of literature is fixated on the same bacteria. Rather than focusing on interventions with limited potential for patient benefit, this would suggest broadening the scope of study to first identify the types of interventions most likely to produce positive effects. Caution is warranted in interpreting the magnitude of effect estimates, given their susceptibility to bias in individual studies and the likely presence of publication bias in the body of literature as a whole.

### Limitations

The primary limitation of this work is likely the high-level evidence synthesis. The standardized mean difference assumes that the same outcome is measured in each study. Many of the tools used to evaluate depressive symptoms assess slightly different facets of the same phenomenon, with substantial overlap. Definitive estimates of efficacy are hindered by heterogeneity of treatment, dosage, study populations and risk of bias. However, a strength of this review is that the tools used to measure outcomes were not part of inclusion criteria; therefore, we captured all validated tools measuring depressive symptoms.

We limited searches to English and French to reduce the number of records screened. Although this strategy may have removed relevant articles in other languages, evidence suggests that language bias does not systematically affect meta-analysis findings beyond reduced precision.<sup>88</sup> Because our objective was to summarize evidence, we elected to stay within the confines of published literature. Therefore, we did not contact authors for studies not presenting sufficient information for inclusion in meta-analysis.

### Conclusion

Our objective was to summarize the effects of interventions targeting gut microbiota on depressive symptoms. This body of evidence is hindered by heterogeneous study quality and the likely presence of publication bias. Although findings are promising, there is not yet strong enough evidence to favour inclusion of these interventions in treatment guidelines for depression. Critical questions about species administered, dosage and timing relative to other antidepressant medications remain to be answered.

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