

Peer-review Comments and Author Responses

Reviewer 1

Introduction

1. The authors have stated their objective in the introduction; however this is not in alignment with the abstract section, which may not be clear for readers. The population seems to be patients with mild to moderate depressive disorder, and this should be clarified and consistent in both sections, as in the main text and conclusion there are statements about major depressive disorder.

Thank you for this insight. We intended to include patients diagnosed with mild, moderate, or severe depression, but our search only yielded studies which included patients with mild and/or moderate depression. We have extended the introductory sentences to more clearly define depression, as well as discuss the influence of the categories of severity. We have also updated the abstract to reflect the changes. “Major depression (MD) is a heterogeneous disorder characterized by persistent low or depressed mood or disinterest in pleasurable activities, in addition to feelings of guilt or worthlessness, fatigue, poor concentration, appetite changes, psychomotor impairment, sleep disturbances, or suicidal thoughts which severely reduce the quality of life (Gutierrez-Rojas et al., 2020; Otte et al., 2016) and is a major risk factor for suicide (Moitra, et al., 2021). The severity of major depression has been associated with short-term treatment outcomes, probability of recovery, and treatment response. The Diagnostic and Statistical Manual of Mental Disorders (DSM) (currently the DSM-V) categorizes the severity of major depression into mild, moderate, or severe strata based on the aggregate of criteria symptoms, the intensity of the symptoms, and the level of functional disability and distress (Kendler, 2016).”

We also amended the results section to clarify this point as follows: “Among the 303 patients (18–65 years) included in the five studies, 217 were female (71.62%). Although we intended to include patients with major depression with mild, moderate, or severe symptoms, our search yielded studies that included patients with mild and moderate symptoms, only.”

2.. Please review reference for Covid-19 and depression

Thank you for pointing out this important oversight. The Santomauro reference has been included in the reference list as follows: “Santomauro, D. F., Mantilla Herrera, A. M., Shadid, J., Zheng, P., Ashbaugh, C., Pigott, D. M., Abbafati, C., Adolph, C., Amlag, J. O., Aravkin, A. Y., Bang-Jensen, B. L., Bertolacci, G. J., Bloom, S. S., Castellano, R., Castro, E., Chakrabarti, S., Chattopadhyay, J., Cogen, R. M., Collins, J. K., ... Ferrari, A. J. (2021). Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. *The Lancet*, 398(10312), 1700–1712. [https://doi.org/10.1016/S0140-6736\(21\)02143-7](https://doi.org/10.1016/S0140-6736(21)02143-7)”.

3. Authors mention tetracyclic antidepressants, serotonin inhibitors but not tricyclics, please review

Thank you for taking the time to address this issue. After careful consideration, we decided to omit the list of examples of types of pharmacotherapies. The sentence now stands as follows: “Treatment options often include psychological or interpersonal therapies in conjunction with antidepressant pharmacotherapies.”

Method

4. In the beginning of the methods section, some clarification would help the reader to understand the nature and PICOS framework, which means detailing the components to which the authors are referring to.

Thank you for this perceptive comment; we have clarified the details of the PICOS and clarified the research question as follows: “The primary research question was: “What is the evidence from the last five years showing the efficacy of probiotics in treating major depression in the general adult population?”The protocol guiding this study shows the PICOS, medical subject headings terms, main concepts retained by each stratum, and search strategy (Caruso et al., 2022). In addition to the PubMed and Web of Science databases used in the reference study (Nikolova et al., 2019), we also searched the Embase database to extend our search results.”

5. It is important to better clarify inclusion and exclusion criteria here.

We fully agree with this suggestion and have added the following statement: “The inclusion criteria were as follows: the population was adults with diagnosed major depression and without neurological and/or psychiatric disorders; the intervention was the use of probiotics (any strain) as an add-on or stand-alone therapy; the comparator was placebo or other treatment; the outcome was an improvement of depressive symptoms; and the study design was randomized controlled trials (RCTs) published between May 2018 and August 2022. To keep the focus of the literature review on the population diagnosed with major depression and to avoid including studies with additional conditions, such as psychiatric disorders, that require in-depth and further analysis, studies that included subjects with neurological disorders or other psychiatric conditions were excluded. Other exclusion criteria included the following: animal studies, case reports, nonprimary research with experimental design, and a lack of focus on major depression.”

6. When authors discuss methodology, it would be advisable to search additional terms to describe exclusions, and maybe consider rephrasing “wrong design/outcome”. According to the authors eligibility criteria, the studies could be excluded if not measuring outcomes of interest, do not report on certain key concepts, etc

We agree with this astute comment and have edited the text in the methods section accordingly. “Three independent reviewers screened the title and/or abstracts of the remaining 108 studies; 85 articles were further excluded because the study design was not an RCT or the intervention was not a probiotic, and 25 were excluded due to lack of focus on major depression. Eight full-text studies were retrieved for evaluation of their eligibility, and a further three studies were excluded: one had an outcome different from depression, one had a nonprimary study design,

and one did not include patients diagnosed with major depression as the study population. Finally, there were five studies included for review.”

Discussion

7. The discussion refers to limitations about generalizability to certain populations and highlights future directions, however the authors should be cautious about the strength of conclusions, as it may lead to the conclusion that there is enough/adequate evidence on the subject.

We agree with this important comment, and we have paid more attention to the limitations of our study so that results would be interpreted within context. This review has some limitations. Our results provide an overall picture of the efficacy of probiotics in the population with mild-to-moderate levels of depression but should be interpreted with caution. First, we included RCTs published in the last 4 years only, which limits generalizability. In this review, we intended to include patients with mild, moderate, and severe symptoms of major depression. However, we did not find studies including patients with severe depression symptoms and thus, the full spectrum of the disorder is not represented in the study population. Even combined, the studies had a relatively small sample size, which also decreases the generalizability of this review. It was also noted that all studies had short follow-up periods, which may limit the likelihood of probiotics reducing symptoms of depression. The concentration and strains of probiotics used in each study were different, which may have introduced treatment heterogeneity and limited the internal validity of the included studies and this review. Furthermore, probiotics were added to a diversity of antidepressants which may have led to population heterogeneity. Finally, we cannot exclude the risk of publication bias because it is likely that studies with negative results have not been published.

We also recommended the following: “[...]. However, the role of probiotics in mitigating depressive symptoms requires phase III research to clarify their effects and allow researchers to develop evidence-based clinical guidelines and inform clinical decision making.”

Conclusion

8. Again, in the conclusion section of the abstract, the authors comment about evidence in mild to moderate depression.

Thank you for this relevant observation. The conclusion was amended as follows: “RCTs published in the last 4 years support the argument that probiotics used as an add-on treatment may have a positive effect in patients with mild-to-moderate symptoms of major depression.”

Reviewer 2

Introduction

9. "Treatment options often include psychological, cognitive behavioral, or interpersonal therapies (...)". Cognitive behavioral is a type of psychotherapy. I would suggest rephrasing it

because it gives the impression that these are the only psychotherapy options. The Division 12 of the American Psychological Association (<https://div12.org/diagnosis/depression/>) provides updated information regarding the evidence of different approaches for specific diagnoses.

This was a very astute comment. To address this point, we exclude cognitive behavioral treatment. We broadly addressed treatment options for depression following the update from The Division 12 of the American Psychological Association (<https://div12.org/diagnosis/depression/>) as follows: “Treatment options often include psychological, cognitive behavioral or interpersonal therapies (...)”.

Method

10. I would suggest clearly stating your research question. You state that you have used the same research question as Nikolova et al. (2019) and that details are in the protocol, but the question is not clearly stated.

Thank you for this comment. We concur that clearly stating the research question would add clarity. Therefore, we added the following statement in the early presentation of methods: The research question was developed in accordance with the systematic review and meta-analysis by Nikolova et al. (2019) and followed the same population, intervention, comparison, outcomes, and study (PICOS) framework (Amir-Behghadami & Janati, 2020). The primary research question was: “What is the evidence from the last five years showing the efficacy of probiotics in treating major depression in the general adult population?”

11. "Studies which included subjects with neurological disorders or other psychiatric conditions were excluded to keep the focus of the literature review on the general population diagnosed with major depression and limit studies with confounding factors or those which may reduce the generalizability of the results". I would suggest rephrasing to be more fluent to read.

Thank you for this suggestion. We edited the phrase to improve readability as follows: “To keep the focus of the literature review on the population diagnosed with major depression and to avoid including studies with additional conditions, such as psychiatric disorders, that require in-depth and further analysis, studies that included subjects with neurological disorders or other psychiatric conditions were excluded. Other exclusion criteria included the following: animal studies, case reports, nonprimary research with experimental design, and a lack of focus on major depression.”

12. "25 were excluded due to wrong outcome. Eight studies were retrieved in full text for evaluation of their eligibility. Three studies were excluded owing to wrong outcome, wrong study design, and wrong inclusion criteria (not depressed patients)". I would suggest rephrasing. The word "wrong" gives the impression that there was actually something wrong with the methodology of these papers, when in reality it was a matter of meeting the inclusion criteria.

We agree with this suggestion and have edited the manuscript as follows: “Eight full-text studies were retrieved for evaluation of their eligibility, and a further three studies were excluded: one had an outcome different from depression, one had a nonprimary study design, and one did not

include patients diagnosed with major depression as the study population. Finally, there were five studies included for review.

Results

13. "Four studies (Kazemi et al, 2019; Schaub et al., 2022; Tian et al., 2022; Majeed et al., 2018) reported significant improvement in depressive symptoms among patients taking probiotics, and one (Rein-ingham et al., 2020) reported significant treatment effect in both placebo and probiotics groups". Do you mean that in the four studies there was a significant improvement compared to placebo?

Thank you for your questions. We clarified the significant improvement compared to placebo reported by four studies as follows: "Patients taking probiotics reported significant improvement in depressive symptoms over the follow-up times (1–3 months) when compared to the placebo groups in four studies (Kazemi et al., 2019; Schaub et al., 2022; Tian et al., 2022; Majeed et al., 2018)."

14. When you say "significant treatment effect in both placebo and probiotics groups", was there any difference between treatment and placebo? The term significant here might be misleading, because if there was no significant difference between probiotics and placebo, then there is no treatment effect, just placebo effect. Since the study included patients newly admitted to hospital, it is possible that the difference from baseline was due to other components of treatment.

Thank you for this important comment. We have amended the results section as follows: "One study reported no significant difference between groups (Reininghaus et al, 2020). At the last follow-up, within-subject improvement was shown in both groups, but the degree of improvement was significantly higher in the experimental arms."

We believe that the improvements in depression symptoms reported in both the treatment and control arms reported by Reininghaus et al. (2020) is worthy of being addressed in the Discussion section as follows: "Notably, one study reported improvements in both probiotic and control groups, showing no significant difference in between group comparisons (Reininghaus et al., 2020). This study was conducted on a sample of newly hospitalized patients with depression. There are several potential reasons for their non-significant results, including (a) limited power to detect effects in newly hospitalized patients, (b) short follow-up period (1 month) which may have been unable to detect add-on effects, (c) sudden change to hospital diet, (d) possible interactions with co-administered biotin and other nutrients, and (e) baseline differences in nutrition and smoking status between groups. There is also a possibility of Berkson's bias because patients were sampled from new hospital admissions, rather than the community. We believe that the non-significant findings of this study are probably due to heterogeneous study methodology rather than different treatment effects."

15. I missed more information on the effect of probiotics found in the included studies. What was the magnitude of improvement found? You said probiotics were an add on treatment, was the baseline treatment similar in both groups? For how long were patients taking the standard treatment? Could the effect be explained by those other treatments?

We fully agree with this important comment. To facilitate the understanding of the magnitude of the effects, we added the Cohen's d calculated from the means, standard deviations, and sample sizes from each study. The Methods section was amended as follows: "For each study, the magnitude of the effect of probiotics on depression compared to the control was summarized using Cohen's d coefficient."

The results section was amended as follows: "Because there were a variety of tools used to assess depression symptoms (Table 1B), we estimated the magnitude of the effects using Cohen's d. The effects of probiotics were large in three studies: Cohen's d coefficients were respectively 1.54 ($p < .001$), 1.17 ($p < .001$), and 0.94 ($p = .005$) in the RCTs reported by Kazemi et al. (2019), Schaub et al. (2022) and Majeed et al. (2018). The effects were moderate (Cohen's $d = 0.64$; $p = .036$) in the study reported by Tian et al. (2022)."

Discussion

16. The first two paragraphs are somewhat repetitive to what has been said in the results section. What was the magnitude of benefit of probiotics? How does it compare to other interventions?

Thank you for this comment. In the first paragraph of the Discussion section, we added a comment on the magnitude of the reported effects and deleted repetitive information as follows: "This systematic mini-review investigated the effect of probiotics on depression by summarizing the results of RCTs investigating probiotics for the treatment of depression, published between May 2018 and September 2022. The aim of the current study was to focus on a population with major depression without any other neurological or psychiatric conditions. For this reason, despite the publication of more recent systematic reviews on broader populations in 2021 (Nikolova et al. 2021), we selected the study by Nikolova et al. (2019) as the launch-point for our own review because it included our specific population of interest. Also, restricting the time period to only studies published from 2021 to 2022 is unlikely to accurately depict the recent state of the evidence. Therefore, we decided to extend the literature search to the publication date of the last primary study included in the Nikolova 2019 systematic review (May 2018). Five studies were included in the review with a combined sample size of 303 patients. All five studies investigated the use of probiotics as an add-on therapy. Each study had a limited sample size (40–110 patients) and a short follow-up period (1–3 months). Four of these studies (Kazemi et al., 2019; Schaub et al., 2022; Tian et al., 2022; Majeed et al., 2018) reported positive findings. The effect magnitudes ranged from moderate (Tian et al., 2022) to large (Kazemi et al., 2019; Schaub et al., 2022; Majeed et al., 2018)."

17. One important point is that you used the systematic review and meta-analysis from Nikolova et al. published in 2019 (which included articles published before May 2018) as a starting point. However, the same group published an update in 2021 (search conducted on 15 May 2020); and El Dib et al. also published a systematic review and meta-analysis including (search conducted on February 10, 2020). Indeed, two of the articles included in you mini-review were already included in these papers (Kazemi et al., 2019 and Majeed et al., 2018). Your mini-review

provides some updates to these previous works, but why didn't you use those most recent articles as a starting point?

Thank you for this important insight. Considering your comment, we have added some considerations to the Discussion to justify our approach. “The aim of the current study was to focus on a population with major depression without any other neurological or psychiatric conditions. For this reason, despite the publication of more recent systematic reviews on broader populations in 2021 (Nikolova et al. 2021), we selected the study by Nikolova et al. (2019) as the launch-point for our own review because it included our specific population of interest. Also, restricting the time period to only studies published from 2021 to 2022 is unlikely to accurately depict the recent state of the evidence. Therefore, we decided to conduct the literature search from the publication date of the last primary study included in the Nikolova et al. (2019) systematic review (May 2018).”

Conclusion

18. "RCTs published in the last 4 years have demonstrated a positive effect conferred by probiotics as an add-on treatment for major depression". I believe we still don't have enough evidence to make such strong conclusions. All the other articles are more careful in their conclusion, stating that probiotics may have a positive effect on depression.

We agree with this important observation and have amended the recommendations in the conclusion section based on the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) Handbook for grading the quality of evidence and the strength of recommendations using the GRADE approach. (Updated October 2013). We assessed the evidence for making clinical recommendations as “weak” under the category of “discretionary (based on opinion of patient or practitioner).” Given the limited sample sizes, the risk of bias, and the short-term follow-up periods of the current evidence, discretion is advised when using probiotics for the treatment of patients with major depression.

General

19. The DOI code is linked to another article

Thank you. This has been addressed.

20. There are some minor diagramation mistakes (words in the same line separated with "-")

Thank you. We believe this was a glitch with auto-correct. It has been addressed.

Reviewer 3

Abstract

21. The statement on the “adequate evidence” might be too certain,

We appreciate this comment and have amended the abstract accordingly. “There is encouraging evidence showing the potential beneficial effect of probiotics as an add-on treatment for patients with major depression with mild-to-moderate symptoms. However, future phase III trials are required to corroborate these results.”

Introduction

22. As the review refers only to the interventions addressed to mild-moderate depression it might be beneficial to include a short description of the characteristics of mild and moderate depression symptoms.

The reviewer has made a valuable point. We intended to include patients diagnosed with mild, moderate, or severe depression, but our search only yielded studies that included patients with mild and/or moderate depression. We included more information about the criteria for depression and provided additional information about the importance of the classification of depression. We also provided a brief description of the classifications as follows: “Major depression (MD) is a heterogeneous disorder characterized by persistent low or depressed mood or disinterest in pleasurable activities, in addition to feelings of guilt or worthlessness, fatigue, poor concentration, appetite changes, psychomotor impairment, sleep disturbances, or suicidal thoughts which severely reduce the quality of life (Gutierrez-Rojas et al., 2020; Otte et al., 2016) and is a major risk factor for suicide (Moitra, et al., 2021). The severity of major depression has been associated with short-term treatment outcomes, probability of recovery, and treatment response. The Diagnostic and Statistical Manual of Mental Disorders (DSM) (currently the DSM-V) categorizes the severity of major depression into mild, moderate, or severe strata based on the aggregate of criteria symptoms, the intensity of the symptoms, and the level of functional disability and distress (Kendler, 2016).”

23. In the Discussion, authors state the important advantages of probiotics, meaning their low-cost and accessibility – this information might increase the relevance if added to the Introduction as well.

Thank you for this helpful comment. We have updated the introduction, stating the advantages of probiotics as follows: “[...]. A stimulating advantage of probiotics is the low cost and accessibility of treatment, which may promote the utilization in clinical practice and optimize the treatment of depression.”

Method

24. Please note that in the Introduction authors state that they eliminate neurological disorders, while in the Materials and Methods section, they also claim to eliminate other psychiatric conditions.

Thank you for pointing out this discrepancy. We edited the introduction by acknowledging that psychiatric diseases were excluded as well as follows: “Using studies published after May 2018, the current systematic mini-review sought to qualitatively summarize the effect of probiotics

(any strain) versus any comparator on depressive symptoms in adults diagnosed with major depression and without neurological and/or psychiatric disorders.”

25. Authors state that, “Studies which included subjects with neurological disorders or other psychiatric conditions were excluded to keep the focus of the literature review on the general population diagnosed with major depression, and limit studies with confounding factors or those which may reduce the generalizability of the results.” - The expression „general population” might suggest all people diagnosed with major depression (like a broader picture), so on the contrary of what authors probably meant. If that is correct, I would suggest deleting „general” and leave the „population diagnosed with”.

We agree with your comment and have amended the manuscript to omit the term “general” as follows: “To keep the focus of the literature review on the population diagnosed with major depression and to avoid including studies with additional conditions, such as psychiatric disorders, that require in-depth and further analysis, studies that included subjects with neurological disorders or other psychiatric conditions were excluded.”

26. Authors mention that they attempt to “[...] limit studies with confounding factors or those which may reduce the generalizability of the results. - I think the concept of the generalizability might be misunderstood. Including papers with possible confounders and comorbidities, like neurological or psychiatric disorders, would not reduce the generalizability - it would increase it.

Thank you for this astute comment. We clarified our purpose in the updated version as follows: “To keep the focus of the literature review on the population diagnosed with major depression and to avoid including studies with additional conditions, such as psychiatric disorders, that require in-depth and further analysis, studies that included subjects with neurological disorders or other psychiatric conditions were excluded. Other exclusion criteria included the following: animal studies, case reports, nonprimary research with experimental design, and a lack of focus on major depression.”

27. Wrong outcome” – I would suggest use of more precise wording, for example „records excluded as outcome did not meet inclusion criteria”.

We agree with this important comment and the text was edited to improve clarity as follows: “Eight full-text studies were retrieved for evaluation of their eligibility, and a further three studies were excluded: one had an outcome different from depression, one had a nonprimary study design, and one did not include patients diagnosed with major depression as the study population. Finally, there were five studies included for review.”

Results

28. Table 1A - Schaub et al., 2022 - lacks the information on female group in the probiotics and placebo arms.

Thank you for this comment. We added the missing information into table 1A.

29. *“Two studies investigated single strains of probiotics (Bifidobacterium breve CCFM1025 and Bacillus coagulans MTCC 5856) and three studies investigated the effect of a blend of probiotics.” - it would be convenient for a reader if these two and three studies are cited in the text.*

We have added citations in the text as follows: “Two studies investigated single strains of probiotics (Bifidobacterium breve CCFM1025 and Bacillus coagulans MTCC 5856) (Majeed et al., 2018; Tian et al., 2022), and three studies investigated the effect of a blend of probiotics (Kazemi et al., 2019; Reininghaus et al., 2020; Schaub et al., 2022). All the blends had at least one strain of Lactobacillus and one strain of Bifidobacterium.”

Conclusion

30. *It was also noted that all studies had short follow-up periods, which may limit their ability to measure the true effect size.” - this sentence sounds like a mental short cut. What does the “true” effect size mean? Authors should revise the sentence and present the thought in a more precise manner.*

This is an important point. We restricted our discussion about specific limitations to the Discussion section. We amended the Discussion section as follows: “It was also noted that all studies had short follow-up periods, which may limit the likelihood of probiotics reducing symptoms of depression.”

31. *In my opinion authors provided a proper explanation of the current state of knowledge and filled the presented gap. Nonetheless, the statement as there is “adequate evidence of beneficial effects of probiotics” might be too certain. Authors should keep in mind that this is a qualitative review of few papers published between 2018 and 2022. Also, some risks of bias were found in the reviewed papers.*

We appreciate this insight and consider this a very important point. After careful consideration we amended our recommendations as follows: “Future phase III studies are needed to corroborate these results and facilitate the development of evidence-based clinical guidelines. Given the limited sample sizes, the risk of bias, and the short-term follow-up periods of the current evidence, discretion is advised when using probiotics for the treatment of patients with major depression.”

General

32. *There are some typos, i.e., „con-trolled”, „de-sign”, „El Dib et al. (2021) conducted a systematic review and meta-analysis focusing on probiotics for the treatment of depression and/or anxiety. and Hofmeister et al. (2021) performed a large meta-analysis of the effects of all interventions targeting gut microbiota.”*

Thank you. We believe this was a glitch with auto-correct. It has been addressed.

Reviewer 4

Abstract

33. In the abstract section, please define the exact period for the articles included in your search.

Thank you for this comment. We have amended the methods section to include a more detailed description of the inclusion and exclusion criteria, including the exact publication period. “A systematic literature search was conducted in PubMed, Web of Science, and Embase databases to identify randomized controlled trials that investigated the effect of any strain of probiotics alone or as an add-on therapy for the treatment of adult patients with mild, moderate, or severe symptoms of major depression and without other neurological and/or psychiatric disorders, published between May 2018 and August 2022. Data were extracted and qualitatively reviewed to determine the treatment effect. The quality of the methodology and risk of bias were assessed using the Cochrane risk-of-bias tool (RoB 2).”

Results

34. In the Methods section, please define the exclusion criteria for those articles that were defined as "wrong" in terms of study design, intervention, or outcome.

We fully agree with this suggestion, and added the following statement: “The inclusion criteria were as follows: the population was adults with diagnosed major depression and without neurological and/or psychiatric disorders; the intervention was the use of probiotics (any strain) as an add-on or stand-alone therapy; the comparator was placebo or other treatment; the outcome was an improvement of depressive symptoms; and the study design was randomized controlled trials (RCTs) published between May 2018 and August 2022.”

Conclusion

35. In the conclusion section, please highlight the limitations of your results and expand on the positive implications of your current findings and future directions. This is a very relevant suggestion.

Thank you. We have added the following statements. “Given the limited sample sizes, the risk of bias, and the short-term follow-up periods of the current evidence, discretion is advised when using probiotics for the treatment of patients with major depression.”

Reviewer 5

Abstract

36. Since it is recommended not to cite in the abstract, is this phrase necessary?

Thank you for this comment. We refined the introduction section to omit any unnecessary information and clearly set the context for the study as follows: “Evidence from randomized

controlled trials investigating the effects of probiotics on depression published in the last four years has not yet been synthesized”.

37. It is not clear if the population included major and minor depression – I believe it should be stated clearly “major depression”

Thank you for pointing out this aspect. We have amended this sentence to specify major depression as: “Evidence from randomized controlled trials investigating the effects of probiotics on depression published in the last four years has not yet been synthesized. The aim of the current systematic mini-review was to summarize the effect of probiotics in adults diagnosed with major depression with mild, moderate, or severe symptoms using studies published after May 2018”.

38. Include the exclusion criteria (time / period of publication); Include the method used to assess risk of bias, to present and synthesize results

We believe this was a very insightful comment. We have completely overhauled the methods section of the abstract and believe that it is more comprehensive and relevant. “A systematic literature search was conducted in PubMed, Web of Science, and Embase databases to identify randomized controlled trials that investigated the effect of any strain of probiotics alone or as an add-on therapy for the treatment of adult patients with mild, moderate, or severe symptoms of major depression and without other neurological and/or psychiatric disorders, published between May 2018 and August 2022. Data were extracted and qualitatively reviewed to determine the treatment effect. The quality of the methodology and risk of bias were assessed using the Cochrane risk-of-bias tool (RoB 2)”.

39. Consider rephrasing without percentage (only 5 studies included)

Percentages have been omitted as suggested.

40. I would write about heterogeneity in the discussion part of the abstract (not in Results), trying to explore it briefly as a limitation of the study

This was a useful suggestion that improved the flow of the section. The Discussion was amended as follows: Despite noted methodological heterogeneity between studies, there is sufficient evidence showing the beneficial effect conferred by probiotics as an add-on treatment for mild-to-moderate depression to substantiate phase III clinical trials in the field.

41. Consider rephrasing – repetition

Thank you for pointing out the repetition. This section was omitted.

42. Since there is no phase 3 RCT, I don't think it is reasonable to think about guidelines yet

This was a key point! Thank you. We have amended our Discussion section to take this important point into consideration as follows: “Discussion: There is encouraging evidence

showing the potential beneficial effect of probiotics as an add-on treatment for patients with major depression with mild-to-moderate symptoms. However, future phase III trials are required to corroborate these results.”

43. Noted issue with inappropriate use of hyphens

Thank you for pointing out the formatting glitch with additional hyphens. We have addressed this concern throughout the manuscript.

Introduction

44. Noted issue with inappropriate use of hyphens (Substances)

Thank you for pointing out the formatting glitch with additional hyphens. We have addressed this concern. We have also made several grammar amendments based on the reviewers’ valuable insights.

Method

45. The inclusion criteria must be explicated (not only written as “the same” of a previous study). Describe the exactly search words and databases used – consider resumming it in the abstract and writing it in details in the methods section

This was a key comment which we believe has added considerably to the replicability of our work. We have updated the methods as follows: “The protocol guiding this study shows the PICOS, medical subject headings terms, main concepts retained by each stratum, and search strategy (Caruso et al., 2022). In addition to the PubMed and Web of Science databases used in the reference study (Nikolova et al., 2019), we also searched the Embase database to extend our search results.”

The inclusion criteria were as follows: “The population was adults with diagnosed major depression and without neurological and/or psychiatric disorders; the intervention was the use of probiotics (any strain) as an add-on or stand-alone therapy; the comparator was placebo or other treatment; the outcome was an improvement of depressive symptoms; and the study design was randomized controlled trials (RCTs) published between May 2018 and August 2022.”

To keep the focus of the literature review on the population diagnosed with major depression and to avoid including studies with additional conditions, such as psychiatric disorders, that require in-depth and further analysis, studies that included subjects with neurological disorders or other psychiatric conditions were excluded. Other exclusion criteria included the following: animal studies, case reports, nonprimary research with experimental design, and a lack of focus on major depression.

The search strategy developed the queries for each stratum of the PICOS, combining strata with the boolean operator AND. The main MeSH terms used for the population were “Adult” and “Depression,” which were searched with a broad combination of synonyms entered as text words

using the boolean operator OR. The main MeSH term for the intervention was “probiotics”, to which 24 additional MeSH indicating the available strains were added by employing the Boolean operator OR. As no specific comparisons were identified, we did not develop a specific query for this stratum to enhance the sensitivity of the final query. The stratum of the outcome was searched by including the MeSH term “Depressive Disorder, Major” and synonyms were added by employing the operator OR. Finally, the clinical query search tool available in the PubMed repository was used to identify RCTs. The final query was adapted from PubMed to the other searched databases.

Results

46. *“Their potential therapeutic applications for the treatment of psychiatric disorders has been tentatively elucidated in pre-clinical and clinical proof-of-concept studies (Schaub et al., 2022; Suneson et al., 2021).” Has -> have.*

Thank you for pointing out these oversights. We have edited the typos and made other grammatical edits based on reviewer E’s suggestions.

47. *“Two studies investigated single strains of probiotics (Bifidobacterium breve CCFM1025 and Bacillus coagulans MTCC 5856) and three studies investigated the effect of a blend of probiotics., All the blends had at least one strain of Lactobacillus and one strain of Bifidobacterium.” place in italics*

Thank you for your suggestion. We have followed the prompt as follows: “Two studies investigated single strains of probiotics (Bifidobacterium breve CCFM1025 and Bacillus coagulans MTCC 5856) (Majeed et al., 2018; Tian et al., 2022), and three studies investigated the effect of a blend of probiotics (Kazemi et al., 2019; Reininghaus et al., 2020; Schaub et al., 2022). All the blends had at least one strain of Lactobacillus and one strain of Bifidobacterium.”

48. *Consider removing the word “wrong” or substituting it; describe the exactly reason for exclusion (e.g.: not RCT, patients with other mental health disorders, etc). Which outcomes the authors considered “wrong”?*

We fully agree with this suggestion. We have edited the text to avoid the term “wrong” by including the precise reasons for exclusions in the Figure and Methods section as follows: “Eight full-text studies were retrieved for evaluation of their eligibility, and a further three studies were excluded: one had an outcome different from depression, one had a nonprimary study design, and one did not include patients diagnosed with major depression as the study population. Finally, there were five studies included for review.”

49. *Italics auto-correct Higgins.*

Thank you for pointing out this oversight. We have updated it.

Discussion

50. *Although it has a small sample size, it is the 2nd largest sample size of the 5 studies*

We have changed “small sample” to “limited power” to detect effects in newly hospitalized patients as follows: “There are several potential reasons for their non-significant results, including (a) limited power to detect effects in newly hospitalized patients, (b) short follow-up period (1 month) which may have been unable to detect add-on effects, (c) sudden change to hospital diet, (d) possible interactions with co-administered biotin and other nutrients, and (e) baseline differences in nutrition and smoking status between groups.”

51. *“The current study has some limitations”. Use “This”*

We have updated the text following your comment as follows: “This review has some limitations.”

52. *I think it should also address Publication bias. Negative studies may not have been published, especially in this field.*

We agree with this insight, and we have updated the text as follows: “Finally, we cannot exclude the risk of publication bias because it is likely that studies with negative results have not been published.”

53. *Parallel-group*

Thank you for pointing out this oversight. The hyphen has been added as suggested.

54. *“Our results provide an overall picture of the efficacy of probiotics in the general population with mild-to-moderate levels of depression, but excluded patients with neurological comorbidities or those with severe diseases.” It was not mentioned earlier in inclusion or exclusion criteria.*

Thank you for this important observation. We have updated the inclusion or exclusion criteria in the current version of the Methods Section as follows: “The inclusion criteria were as follows: the population was adults with diagnosed major depression and without neurological and/or psychiatric disorders; the intervention was the use of probiotics (any strain) as an add-on or stand-alone therapy; the comparator was placebo or other treatment; the outcome was an improvement of depressive symptoms; and the study design was randomized controlled trials (RCTs) published between May 2018 and August 2022.”

To keep the focus of the literature review on the population diagnosed with major depression and to avoid including studies with additional conditions, such as psychiatric disorders, that require in-depth and further analysis, studies that included subjects with neurological disorders or other psychiatric conditions were excluded.

55. *“Our results provide an overall picture of the efficacy of probiotics in the general population with mild-to-moderate levels of depression, but excluded patients with neurological comorbidities or those with severe diseases.” Not mentioned before in inclusion and exclusion*

criteria – although written before that the remain 5 studies only included mild-to-moderate, I think it should be better highlighted once again here in the discussion

Thank you for this suggestion. We have updated the manuscript as follows: “In this review, we intended to include patients with mild, moderate, and severe symptoms of major depression. However, we did not find studies including patients with severe depression symptoms and thus, the full spectrum of the disorder is not represented in the study population.”

56. “Healthcare providers may benefit from clinical guidelines with an evidence-based framework to inform the use of probiotics as an adjuvant therapy for improving the quality of life of patients with depression.” I think it should be rephrased, so that it does not seem that the authors are recommending to include probiotics in clinical guidelines since no phase III RCT has been published yet.

This is a very insightful and valuable comment. After careful consideration, we have amended our recommendations as follows: “An exciting advantage of probiotics is the low cost and accessibility of treatment, which may facilitate broad utilization in clinical practice and optimize the treatment of depression worldwide. However, the role of probiotics in mitigating depressive symptoms requires phase III research to clarify their effects and allow researchers to develop evidence-based clinical guidelines and inform clinical decision making.”

Conclusion

57. “RCTs published in the last 4 years have demonstrated a positive effect conferred by probiotics as an add-on treatment for major depression.” Generalization: if only mild-to-moderate patients with major depression were included in this review, it is not possible to conclude that, especially for patients with major depression with severe symptoms.

Thank you for this suggestion. The conclusion was amended as follows: “RCTs published in the last 4 years support the argument that probiotics used as an add-on treatment may have a positive effect in patients with mild-to-moderate symptoms of major depression.”

58. “However, future empirical studies and comprehensive systematic reviews with meta-analyses are needed to address uncertainties and consolidate the available evidence in the field.” Should a phase 3 RCT be suggested instead of empirical studies? So that it can be practice changing? I don’t think it is an adequate conclusion since no phase III RCT has been published yet.

This was a very important comment. Thank you. We have edited the text as follows: “ Future phase III studies are needed to corroborate these results and facilitate the development of evidence-based clinical guidelines.”